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INHERITANCE OF SEED WEIGHT IN GREEN GRAM (PHASEOLUS AUREUS ROXB)

NIRAD K. SEN AND A. S. N. MURTY

Applied Botany Section, Indian Institute of Technology, Kharagpur, India

Received August 9, 1960

GREEN gram (*Phaseolus aureus*) and black gram (*Ph. mungo*) are two important legumes of India and Far East, which are grown principally for their protein rich seeds. Evidence suggests their origin in north eastern India, where most of the wild Phaseolus are small seeded. Inheritance of seed size was studied in crosses among the small, medium and the big-seeded agricultural varieties of green gram to get information about the factors contributing to the increase in seed weight.

MATERIALS AND METHODS

In our genetical stocks of green gram (SEN and GHOSH 1959), the variety Sonamung has the smallest seed, BR 3 and NP 36 represent the medium group, and the varieties Brown fruit and EB 6 have big seeds. In the different cross combinations made among the small, medium and the big-seeded varieties, the parents, F_1, F_2, and F_3 progenies were grown in rows in adjacent blocks, with the two parents and the F_1 in the middle row, the F_2 plants on their two sides followed by the rows of the F_3 plants. For the parents and the F_1 progeny 10–20 plants as available were taken, there being 150–250 F_2 plants, and 300–400 F_3 plants. To sow the seeds of the four generations at the same time, seeds of the two parent types and the F_1 plants were collected, and fresh crosses were made in the previous year. The F_3 plants represent progeny of randomly selected F_2 plants, which were also raised in the year before. At maturity the ripe pods were hand-picked and sun-dried, and 50 to 100 seeds as available were weighed rejecting the deformed and the shrunken ones. The seed weight of the plants has been expressed as the weight per seed in milligram.

OBSERVATIONS

The seed weights of the parents and their F_1, F_2 and F_3 progenies with their range, mean, standard deviation and standard error in the different cross combinations are given in Table 1.

In the cross between the small × medium seeds, the F_1, F_2 and F_3 means are very close to the small parent. The range of F_1 seeds was within that of the small parent, those of the F_2 and F_3 progenies extended beyond it, but seeds as large as the largest seed of the medium parent were not recovered. In the small × big cross, the difference between the mean seed weight of the two parents was about five times more than in the small × medium cross. Even then the F_1 mean was

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TABLE 1

Seed weight in milligrams in parents, F_1, F_2, and F_3 generations of different crosses

Cross	Generation	Range	Mean	S.D.	S.E.	C.V.
Small	P_1 Sonamung	15–25	20.95	3.10	1.265	14.79
×						
Medium	P_2 BR 3	18–37	28.31	5.18	0.979	18.30
	F_1	18–25	21.45	1.75	0.877	8.15
	F_2	12–34	21.83	3.45	0.244	15.81
	F_3	12–34	21.85	2.84	0.123	13.00
Small	P_1 Sonamung	15–25	20.94	3.04	1.265	14.80
×						
Big	P_2 EB 6	57–64	59.45	2.07	0.841	3.48
	F_1	18–25	20.45	2.69	1.633	13.15
	F_2	12–37	22.18	4.58	2.272	20.64
	F_3	15–52	30.17	6.92	0.137	22.93
Medium	P_1 BR 3	18–34	28.31	5.18	0.98	18.31
×						
Medium	P_2 NP 36	18–31	24.45	4.66	1.14	19.06
	F_1	18–22	20.45	1.12	0.646	5.46
	F_2	12–37	25.30	3.73	0.22	14.78
	F_3	18–40	25.42	2.90	0.27	15.31
Big	P_1 Brown fruit	43–55	49.45	4.15	1.43	8.39
×						
Big	P_2 Chinimung	49–71	60.95	6.04	1.44	9.91
	F_1	37–56	45.70	5.49	1.94	12.01
	F_2	25–86	43.54	10.42	1.07	23.93

very close to the small parent. The F_2 mean, however, shifted towards the big parent and the F_3 mean shifted still further, being more or less at the mid-point of the difference between the small parent and the arithmetic mean of the two parents. The range of the F_1 seeds was within that of the small parent, and as in the small × medium cross, plants with seeds smaller than those of the small-seeded parent were recovered, but none were recovered with seeds larger than that of the big parent.

In both the crosses between the two medium-seeded and the two big-seeded varieties, the F_1 mean was smaller than their small parent, showing a negative heterosis. The F_2 and the F_3 frequencies were positively skewed, and the two means were closer to their small parent. In these two crosses not only seeds smaller than the small parent were recovered, but also plants with seeds bigger than the large parent were recovered.

DISCUSSION

The results of the two crosses of the small-seeded Sonamung with the medium-seeded BR 3 and the big-seeded EB 6 indicate that the small seed character is more or less completely dominant. The most simplified hypothesis for the origin of the larger seeded types from the small-seeded ones with seeds similar to the wild type Phaseolus is through accumulation of recessive mutations of positive value. If bigger size of the seed has no selective disadvantage in nature, these genes contributing to the seed weight will accumulate, and men might have exerted selection pressure to evolve better seeds. The plants with bigger seeds are likely to have more of these genes contributing to the seed weight than the medium-seeded type.

The results also indicate that though both the medium varieties arose through the accumulation of recessive genes contributing to the seed weight they have different sets of these genes. In the hybrid, as these genes for the two parents are dominated by the wild type genes, their contribution to increased seed weight is nullified, and the F_1 mean shifts beyond the small parent showing negative heterosis in POWERS' (1944) scale. As expected from such a setup, in some plants of the segregating generation recombination has brought in the recessive contributing genes of both the parents resulting in recovery of seeds bigger than the largest seeded parental plants. The observed results of the cross between the two big-seeded varieties are also similar and can also be explained in this way.

Inheritance of seed weight in green gram is likely to be much more complicated than the simplest model assumed to interpret the observed results. But the recessive nature of most of the genes contributing to the seed weight is likely to be predominant. As different genes may accumulate in varieties with similar seed weight. there may be a possibility of increasing seed weight in green gram by crossing among the different big-seeded varieties and picking up the recombination types with still bigger seeds as seen in the cross between the two big-seeded varieties.

SUMMARY

In crosses among the small, medium and big-seeded varieties of green gram, the small seed is seen to be more or less completely dominant over the medium and the big seeds. In the F_2 and F_3 progenies, seeds smaller than the parents could be recovered but not plants with seeds even as large as the existing ones in the larger parent. In the crosses between the two medium-seeded and also between the two big-seeded varieties, the hybrid showed negative heterosis. In both the crosses, seeds much smaller than the parental smallest seeded plant and seeds larger than the largest seeded parental plants were recovered in the segregating generations. It has been postulated that in green gram the medium and the big-seeded varieties have evolved from the small-seeded types through the accumulation of recessive genes contributing to seed weight with additive effect. The results indicate that by crossing among the different big-seeded varieties, it will be possible to improve further the seed weight in this plant.

ACKNOWLEDGMENT

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LITERATURE CITED

Powers, L., 1944 An expansion of Jones' theory for the explanation of heterosis. Am. Naturalist **78**: 275–280.

Sen, N. K., and A. K. Ghosh, 1959 Genetic studies of green gram (*Phaseolus aureus*). Indian J. Genet. and Pl. Breed. **19**: 210–227.

PHENOGENETICS OF THE LOZENGE LOCI IN DROSOPHILA MELANOGASTER. I. EFFECTS OF A SUPPRESSOR OF lz^{34k}

HARVEY A. BENDER[1,2] AND M. M. GREEN

Department of Entomology and Parasitology, and Department of Genetics, University of California, Berkeley and Davis

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ABNORMALITIES of the accessory female genitalia and an accompanying decrease in fertility were noted in the logenze (*lz*) group of mutants of *Drosophila melanogaster* (OLIVER and GREEN 1944). The correlation between reduced female fertility and the absence of the spermathecae in individuals homozygous for these mutants led to the suggestion of the need of these structures in Drosophila for high and continued fertility (ANDERSON 1945). However, the absence of spermathecae cannot wholly account for the reduced fertility of *lz* females since variations in fertility were observed among several independent *lz* mutants as well as their compounds all lacking spermathecae (OLIVER and GREEN 1944).

The finding of a suppressor mutation which alters the eye phenotype of the mutant lz^{34k} motivated a detailed re-examination of the causes of reduced fertility associated with homozygous *lz* females.

MATERIALS AND METHODS

Phenotypic effects associated with the sex-linked, recessive *lz* mutants include: modification of eye color, structure and size, absence of spermathecae and pars ovariae, and reduced tarsal claws. In a stock culture of double-X, yellow bodied and forked winged females and lz^{34k} males two types of males were found. Some males were typically lz^{34k} in phenotype; others had distinctly more normal eyes which are larger, more normal in coloration and less "rough" in appearance. Genetic analysis showed that the atypical phenotype was causally associated with a recessive suppressor mutation localized in the third chromosome. This suppressor mutant is designated $su(3)lz^{34k}$. Subsequent observations showed that the fertility of females homozygous $lz^{34k};su(3)lz^{34k}$ was distinctly greater than that of lz^{34k} females, and an unbalanced stock $lz^{34k};su(3)lz^{34k}$ could be maintained. On examination it was found that spermathecae and pars ovariae were missing in homozygous $lz^{34k};su(3)lz^{34k}$ females just as in homozygous lz^{34k} females. The absence of spermathecae in both classes of females suggested that the increased fertility of the suppressed females could not be accounted for solely by this phenotypic abnormality. Therefore a comparative study of fertility and histology of the female reproductive organs was undertaken.

[1] Public Health Service Research Fellow of the National Cancer Institute.

[2] Present Address: Department of Biology, University of Notre Dame, Notre Dame, Indiana.

Where female fertility comparisons were made, the following procedure was adopted. Virgin females homozygous lz^{34k}, lz^{34k};$su(3)lz^{34k}$, and control (lz^{34k}/Basc) were mated individually to two males. The flies were grown in creamers and/or vials containing corn meal-agar culture media darkened with charcoal when egg counts were made. Egg production of the females was observed over a two-week period following eclosion. Fertility is here defined as the production of any hatchable eggs and fecundity as the number of hatchable eggs. All flies were grown at the laboratory temperatures of 23±3°C.

Feulgen-prepared whole mounts and stage distribution analysis of the ovarian tissue of the three classes were obtained following the techniques of KING, RUBINSON and SMITH (1956).

RESULTS

The data on fertility and fecundity are recorded in Table 1. Females were mated either to wild type, lz^{34k}, or lz^{34k};$su(3)lz^{34k}$ males. In column two the number of matings with each type of female is tabulated. The column marked "fertility" denotes for each genotype the number and percent of viable females producing offspring. In the column marked "fecundity" the mean number of offspring produced per fertile female of each genotype is given together with its standard error. These data strikingly show the low proportion of fertile lz^{34k} females and their relatively poor fecundity. The presence of the suppressor increases fertility by more than threefold, the fecundity by over 28 percent. The fecundity of the suppressed lz^{34k} females is, however, still significantly below that of the controls. The viability, measured as the percent of tested P_1 females living on the fourth day, was not significantly different among the three classes. More than 90 percent of the females of each genotype were viable.

Daily egg-hatchability was measured over a two-week interval for females of all three genotypes to determine whether the low fertility and fecundity of the lz^{34k} females was due to low egg production. The results are given in Table 2. Egg production is drastically reduced in the lz^{34k} females as compared with the controls. The suppressed lz^{34k} females are intermediate. Egg-hatchabilities of 14, 35, and 73 percent were observed in the lz^{34k}, suppressed, and control groups, respectively. Sterile lz^{34k} females could be divided into two groups: those laying eggs which failed to hatch and those which failed to lay any eggs whatsoever.

Analysis of the developing oocytes of the lz^{34k} females demonstrates an abnormally high number of yolk-filled stages by the fourth day, and this condition

TABLE 1

Fertility and fecundity of lz^{34k} *and suppressed* lz^{34k} *females*

Females genotype	No. females mated	Fertility No.	Fertility Percent	Fecundity Mean	Fecundity S.E.
lz^{34k}	122	26	21	28	6.8
lz^{34k};$su(3)lz^{34k}$	183	142	77	36	1.0
lz^{34k}/Basc	87	72	83	58	3.2

TABLE 2

Egg production and hatchability of lz^{34k}, *suppressed* lz^{34k} *and control females**

Day	lz^{34k} Eggs/female	lz^{34k} Hatched eggs/female	lz^{34k};su(3)lz^{34k} Eggs/female	lz^{34k};su(3)lz^{34k} Hatched eggs/female	lz^{34k}/Basc Eggs/female	lz^{34k}/Basc Hatched eggs/female
1	0	0	0	0	0	0
2	1.5	0.3	1.3	0.3	0.3	0.1
3	3.0	0.1	12.5	2.3	4.8	2.5
4	2.0	0.3	15.5	11.0	20.3	17.3
5	4.3	0.7	9.5	5.3	27.5	25.0
6	1.5	0.3	7.8	5.8	19.0	16.8
7	0	0	10.0	1.8	20.3	20.0
8	0	0	6.5	1.0	10.0	7.5
9	0	0	4.0	1.5	12.0	9.8
10	0	0	4.3	0.3	4.5	2.5
11	0	0	2.0	1.8	13.3	8.3
12	0	0	13.0	1.8	9.0	2.3
13	0	0	11.3	4.5	19.3	11.0
14	0†	0	5.0	1.0	13.3	3.3

* These data are pooled from observations of two replicate experiments utilizing four individual females in each of the three classes per replication.
† One lz^{34k} female laid one sterile egg on the 14th day.

becomes progressively more pronounced with age. Complete serial sections of paraffin-embedded female reproductive systems demonstrate that the yolk-filled follicles of many of the ovarioles degenerate by the seventh day, and the lumina of the reproductive ducts fill with what appears to be necrotic yolk material. The suppressed females display a similar array of pathologies which, however, are delayed in degree and time of onset. The details of these histo-pathologies are to be reported elsewhere.

DISCUSSION AND CONCLUSIONS

This study demonstrates that high and continued fertility can be re-established in the lz^{34k} females through the utilization of a suppressor mutation. The low fertility of the lz^{34k} females is shown primarily to be attributable to ovarian abnormalities and secondarily to the lack of the spermathecae. Further insight into the underlying causes of reduced fertility associated with lz^{34k} females stems from a preliminary transplantation experiment. In this study lz^{34k} ovaries transplanted into wild-type hosts manifest a marked increase in fertility (CLANCY, personal communication). This nonautonomous behavior suggests that circulating substance(s) in the wild-type female hosts promote the more normal development of the lz^{34k} ovary. From this fact it is possible that the suppressor mutation acts to promote more normal ovarian development in lz^{34k} females in a manner comparable to the development of lz^{34k} ovaries in wild-type hosts.

SUMMARY

(1) Increased fertility can be established in lz^{34k} females by means of an autosomal suppressor mutation. (2) The low fertility of lz^{34k} females is shown to be

primarily due to ovarian abnormalities and secondarily to the lack of accessory reproductive structures.

LITERATURE CITED

ANDERSON, RAY C., 1945 A study of the factors affecting fertility of lozenge females of *Drosophila melanogaster*. Genetics **30**: 280–296.

KING, R. C., A. C. RUBINSON, and F. R. SMITH, 1956 Oogenesis in adult *Drosophila melanogaster*. Growth **20**: 121–157.

OLIVER, C. P., and M. M. GREEN, 1944 Heterosis in compounds of lozenge alleles in *Drosophila melanogaster*. Genetics **29**: 331–347.

EFFECTS OF SOME AUTOSOMAL INVERSIONS ON LETHAL MUTATION IN DROSOPHILA MELANOGASTER[1, 2]

PETER E. THOMPSON

Department of Zoology, University of Texas, Austin, and
Department of Zoology, University of California, Berkeley

Received October 26, 1959

WITH the development of genetics, it has become implicit in the principle of segregation that the purity of segregating alleles or homologous chromosomes is subject to exception only where mechanisms of recombination are concerned. Recently, cases of apparent interallelic modification have been described where thorough analysis has precluded the possibility of a basis in any recognized mechanism of recombination. Probably the most striking of these is BRINK's (1958) observation in maize that a factor for self-colored aleurone, R^r, when paired with a stippled allele R^{st}, invariably changes to a stable and heritable factor producing a weakly pigmented aleurone. The consistent alteration of the R^r allele does not fit into the pattern of random exchange associated with crossing over. There is good evidence also that no modifiers outside the R locus are responsible for the phenomenon.

STURTEVANT (1939) has obtained evidence suggesting a more general mutative interaction of homologues. He observed that hybrids between two semicross-sterile races of *Drosophila pseudoobscura* show a large increase in mutation frequency over either parental race. He interpreted this increase as a release of variability due to the upsetting of a balanced physiological environment. These races, frequently classed as separate species (*D. pseudoobscura* and *D. persimilis*), differ constantly in chromosomal arrangement by one inversion in the left arm of the X chromosome and one inversion in chromosome II, usually differ by an inversion in the right arm of the X chromosome, and may have other differences depending on intraspecific variation (DOBZHANSKY and EPLING 1944). GOLDSCHMIDT (1955) has recently attributed the increase in mutation noted by STURTEVANT to stress resulting from imperfect synapsis in structurally different homologous chromosomes.

The present study was stimulated by an observation made while working with DR. A. B. BURDICK at Purdue University, using marked multiple-inversion chromosomes and a system of inbreeding to produce isogenic lines for analyses of quantitative inheritance. It was expected, in view of their theoretical homozygosity, that the isogenic lines obtained by this procedure would be lethal free. When such lines had been obtained, it was found convenient to repeat the process

[1] Taken in part from a dissertation submitted to the Graduate School, University of Texas, in partial fulfillment of the degree of Doctor of Philosophy, 1959.

[2] A portion of this investigation was carried out during the tenure of a Postdoctoral Fellowship from the National Heart Institute, United States Public Health Service.

of "isogenization" on these lines rather than use fresh wild sources. The immediate repetition of the extraction process revealed that an unexpectedly high proportion of lines were already heterozygous for recessive lethals carried on the chromosome presumed to be isogenic.

Because of the frequent occurrence of lethals in chromosomes II and III, an explanation was sought in the genetic manipulation of these normal autosomes, particularly their association with multiple-inversion chromosomes. Systematic comparisons of lethal mutation rate were made for multiple-inversion heterozygotes and structurally homozygous controls.

RESULTS AND DISCUSSION

Inversion heterozygosis and lethal mutation rate: Estimates of lethal mutation frequency in normal chromosomes paired with multiple-inversion chromosomes, and in normal chromosomes made completely homozygous or isogenic, were carried out in *Drosophila melanogaster*. The normal chromosomes derived from several wild sources: Erie, Espanola, N. M., Oregon-R, and Canton, and the background of a laboratory stock of Muller-5, a complex of X chromosome mutants. The procedure outlined in Figure 1 was used to obtain isogenic, lethal free individuals (class "a") in which to test mutation as a control, and to obtain at the same time structurally heterozygous siblings (class "d") of these flies in which to

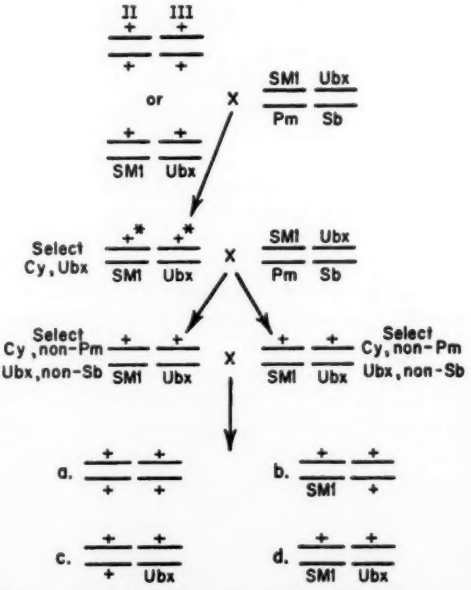

FIGURE 1.—Mating scheme for the formation of experimental lines, and for the detection of autosomal lethals. Asterisks indicate chromosomes in which the presence of lethals is being tested.

test mutation experimentally. This procedure was carried out for chromosomes II and III simultaneously by the use of a stock having the multiple-inversion chromosome Second Multiple 1 (*SM1*) and its associated marker Curly (*Cy*) balanced with the Plum marked inversion (*Pm*), and the multiple inversion Ultrabithorax-130 (Ubx^{130}) balanced with Stubble (*Sb*). Each of these marked chromosomes is lethal wihen homozygous, so that the genetic constitution at each stage of the procedure may be readily discerned.

The test of the production of new lethals in selected isogenic or heterozygous parents involved the same procedure as the extraction of these progenitors (Figure 1), being based on the elimination of isogenic classes where lethals are present in the tested chromosomes (asterisks, Figure 1). The multiple-inversion chromosomes with which the normal chromosomes were paired in heterozygous progenitors were *SM1*, having six breaks in chromosome II (LEWIS and MISLOVE 1953), and Ubx^{130} with five breaks in chromosome III (LEWIS 1952). These are the same inversions which were used in the Purdue isogenic extractions.

From every initial test mating several progeny were selected, each of which carried one normal-II and one normal-III chromosome (asterisks). Each such offspring represented one gametic set of normal, or possibly lethal bearing, autosomes sampled from the tested parent. A means was at hand for distinguishing the mode of origin of lethals by the manner of their occurrence. A lethal arising during meiosis is recovered in only one of several gametes sampled from the progenitor. Lethals from a single premeiotic event in the germ line are often recovered in a cluster of several gametes out of a large number (CASPARI and STERN 1948; SPENCER and STERN 1948), which prove functionally allelic when tested for lethality in combination. A preexistent lethal is recovered in all gametes sampled from a heterozygous individual. In all of the present work only the production of fully lethal factors was considered. These were scored as instances where a terminal progeny of at least 100 flies consisted of all Curly or all Ultrabithorax; furthermore each lethal maintained itself as a balanced stock for two additional generations.

Comparisons of spontaneous mutability were carried out from June, 1957 to March, 1959. In a majority of cases the progenitors were male, although in several of the first series both male and female progenitors were used. An early comparison showed that no significant difference existed between sexes ($P = .76$ for Espanola, Erie 1957 and Oregon-R groups). Thereafter, for convenience in culturing, only male parents were used.

A comparison of the frequency of chromosome-II lethals in isogenic and heterozygous sources is given in Table 1. A similar comparison for chromosome III is given in Table 2. The difference in frequency between the two chromosomes is not significant ($P = .45$) suggesting that the observed effect of heterozygosity is not uniquely associated with *SM1* or with Ubx^{130}. There is no evidence of heterogeneity among sources for the isogenic group ($.80>P>.50$) or for the heterozygous group ($.95>P>.50$), and a compilation of total lethal frequencies in control and heterozygous groups is presented in Table 3.

The compiled data of Table 3 were used to estimate the significance of the ob-

TABLE 1

Occurrence of lethals in chromosome II

Source of wild chromosomes	Sex tested	Isogenic +/+;+/+ Chromosomes tested	Isogenic +/+;+/+ Lethals Meiotic	Isogenic +/+;+/+ Lethals Premeiotic	Heterozygous *SM1/+;Ubx/+* Chromosomes tested	Heterozygous *SM1/+;Ubx/+* Lethals Meiotic	Heterozygous *SM1/+;Ubx/+* Lethals Premeiotic
Espanola	♂	72	0	0	28	1	0
Espanola	♀	115	0	0	139	1	0
Erie (1957)	♂	52	1	0	12	0	0
Erie (1957)	♀	12	0	0	81	1	0
M-5 background	♂	37	0	0	68	1	0
Oregon-R	♂	43	0	0	106	1	0
Oregon-R	♀	54	0	0	125	0	0
Erie (1958)	♂	151	0	0	599	7	2
Canton	♂	42	1	0	185	4	0
Total		578	2	0	1,343	16	2

TABLE 2

Occurrence of lethals in chromosome III

Source of wild chromosomes	Sex tested	Isogenic +/+;+/+ Chromosomes tested	Isogenic +/+;+/+ Lethals Meiotic	Isogenic +/+;+/+ Lethals Premeiotic	Heterozygous *SM1/+;Ubx/+* Chromosomes tested	Heterozygous *SM1/+;Ubx/+* Lethals Meiotic	Heterozygous *SM1/+;Ubx/+* Lethals Premeiotic
Espanola	♂	72	0	0	28	0	0
Espanola	♀	115	0	0	139	1	0
Erie (1957)	♂	52	0	0	12	0	0
Erie (1957)	♀	12	0	0	81	1	0
Oregon-R	♂	43	0	0	106	1	0
Oregon-R	♀	54	0	0	125	0	0
Erie (1958)	♂	151	1	0	599	6	1
Canton	♂	42	0	0	185	3	0
Total		541	1	0	1,275	12	1

TABLE 3

Total lethal frequencies: control vs. *experimental*

	Isogenic +/+;+/+ Chromosomes tested	Isogenic +/+;+/+ Lethals Meiotic	Isogenic +/+;+/+ Lethals Premeiotic	Heterozygous SM1/+;Ubx/+ Chromosomes tested	Heterozygous SM1/+;Ubx/+ Lethals Meiotic	Heterozygous SM1/+;Ubx/+ Lethals Premeiotic
II	578	2	0	1,343	16	2
III	541	1	0	1,275	12	1
Total	1,119	3	0	2,618	28	3
Lethal percentages		0.27	0		1.06	0.11

served difference in frequency of meiotic lethals between isogenic and heterozygous sources. The appropriate statistic for such a binomial population is $u = \bar{y}_{het} - \bar{y}_{iso}/\sqrt{\bar{\bar{y}}\ (1-\bar{\bar{y}})\ (1/n_{het} + 1/n_{iso})}$ (LI 1957). The calculated value of u is 2.48, a value having a probability of .0068. The significance is still greater if this is considered to be a one-tailed test, which it is. That is, the aim of the experiment was not merely to demonstrate that the groups differed, but the experiment was designed to test specifically whether or not the lethal mutation frequency was higher in the heterozygous than in the isogenic group. The fact that the observed difference was in the right direction adds to the significance of the finding.

Tests of heterologous effects: With the demonstration that an increase in autosomal mutability is associated with heterozygosity for *SM1* and *Ubx*^{130}, it became a matter of interest whether the sites of mutation follow a pattern of randomness like that associated with IVES' high (*hi*) factor (IVES 1950). In the case of *hi* the mutability factor is a point mutant in chromosome II, but it was found that when the mutant is homozygous not only is the over-all mutation rate in chromosome II greatly increased, but also mutability in the heterologous X chromosome undergoes a comparable increase. It has been proposed that the effect of *hi* is mediated by an alteration in cytochemical milieu, which accounts for the generality of effect.

A comparison of the rate of incidence of sex-linked lethals was made in F_1 males from a cross of Canton females and *SM1*/+;*Ubx*^{130}/+ males. The control group had no heterologous inversions; the experimental group carried *SM1* and *Ubx*^{130} among its autosomes. As seen in Table 4, the change in frequency of X chromosome lethals when the heterologous inversions are or are not present is not even in the same direction as the fourfold increase obtained in the autosomes when *SM1* and *Ubx*^{130} are present. In this respect, then, the mutability associated with *SM1* and *Ubx*^{130} is dissimilar to the very generalized mutability of IVES' *hi* factor or MAMPELL's (1945) mutator in *D. persimilis*, and it seems unlikely that the mechanism is one of indiscriminate cytochemical activity.

Synergistic response to irradiation: A class of mutability phenomena exists which is characterized by localized effects and has a basis in the cycle of chromosomal changes, synapsis, and related chemico-mechanical events. This seems a natural category for BEADLE's (1932) sticky factor, the Dissociation and Activator factors (MCCLINTOCK 1951), and Dotted (RHOADES 1941), all in maize. The case of "paramutation" in maize reported by BRINK (1958) may have a similar basis, as well as the segregation distortion in Drosophila reported on by SANDLER *et al.* (SANDLER, L., HIRAIZUMI, and I. SANDLER 1959; SANDLER and HIRAIZUMI

TABLE 4

The effect of SM1 *and* Ubx^{130} *on X chromosome mutation rate*

Genotype	Chromosomes	Lethals	Percentage
+/Y;*SM1*/+;*Ubx*/+	1,310	8	0.61
+/Y;+/+;+/+	1,181	8	0.68

1959). No precise genetic or cytogenetic method for establishing a role of pairing relationships in genic alterations at meiosis has yet been described. Nevertheless, NOVITSKI (1946) has convincingly employed a "synaptic stress" hypothesis in reconstructing the phylogeny of a series of nearly identical chromosomal rearrangements in *D. athabasca*. He attributes the occurrence of new, non randomly localized inversion breaks to the unsatisfied and conflicting forces of synapsis adjacent to the break points of existing inversions.

The degree of structural heterozygosity in the lines reported on has suggested that an effect of the sort hypothesized by NOVITSKI might be in operation, with chromosomal disruption reflected as recessive lethality rather than inversions.

A related possibility is that the unpaired regions adjacent to break points in inversion heterozygotes have an increased mutability by virtue of their asynapsis, at least during critical stages of meiosis. One might suppose that despiralization or the removal of matrix during this time creates an alterability which is normally offset by greater stability of the paired condition. This supposition receives some support from the observation of AUERBACH (1941) that the unpaired X chromosome of Drosophila males undergoes much more spontaneous mutation than the paired X chromosomes of females.

Either of these hypotheses might be tested against the existence of a cytochemical agent of mutation by determining the level of mutation in heterozygotes subjected to treatment with an exernal agent. If the basis of the observed mutability effect were an internal agent of mutation, such as one or more cytochemical mutator substances, purely additive effects of internal and external agents would be anticipated. This is clear from the failure to find synergistic increases in the level of mutation from ionizing radiations combined with a variety of chemical or physical agents, excepting those which affect the ease with which broken ends of chromosomes reattach (MULLER 1954). The latter would not have any appreciable effect without additional treatment and do not in themselves qualify as mutator substances.

It may be seen that under the hypothesis of stress or mutability adjacent to inversion break points the effect is likely to be one of less ready restitution when a local alteration of the linearity of components occurs. Under this hypothesis, the local response to irradiation would be as strong as by the use of a chemical which prevents the reunion of chromosomal breaks, ionization and asynapsis producing a high level of mutation in synergistic fashion.

As a test of this hypothesis males of the constitutions *SM1*/+;*Ubx*^{130}/+ and +/+;+/+ were irradiated with 1000r of X-rays and lethal detection procedures were carried out for chromosomes II and III as previously described. The normal chromosomes derived from a Canton-S stock and the irradiated males were treated 0–2 days after eclosion. Each such male was mated to separate pairs of females at two-day intervals to sample sperm over six mating periods, from 0–12 days. Since sperm sampled in the first two matings were either mature or in the spermatid stage at the time of treatment and presumably would not show sensitivities related to the hypothesized synaptic properties, the females employed during these periods were discarded and served only to collect and remove mature sperm.

The incidence of autosomal lethals from these irradiated sources is summarized in Table 5. Probabilities were estimated by tests of chi-square with COCHRAN's (1942) adjustment for small numbers. During every mating period the frequency of both chromosome-II and chromosome-III lethals was higher in the structurally heterozygous lines than in the isogenic lines, and the difference in total frequencies for each linkage group was significant. This difference was most pronounced at 6 to 8 and 8 to 10 days, although sterility in the latter period detracts from its statistical significance. Extensive genetic analysis (AUERBACH 1954) and cytological evidence (WELSHONS and RUSSELL 1957) establishes the correspondence of these periods to late spermatogonial and meiotic stages. This is in line with the occurrence of sterility at 8–10 days which WELSHONS and RUSSELL have demonstrated is due to the killing of secondary spermatogonia and young spermatocytes. Thus, beyond demonstrating an increase in lethal frequency, the brood pattern method shows that the greatest increase occurs at a time when the synapsis of homologous chromosomes is most intimate, judging by the evidence from induced crossing over (AUERBACH 1954).

The most striking aspect of these data, however, is that the increase seen in meiotic stages is considerably more than the sum of the high spontaneous mutability (1.06 percent) seen in the initial use of inversion heterozygotes and the normal response to 1000r (7.0 percent at 6–8 days or 6.6 percent at 8–10 days) seen in the control. It is, rather, a highly exaggerated effect which points to synergistic interaction of radiation-induced chromosomal alterations and the

TABLE 5

Lethal frequencies in structurally heterozygous and homozygous lines after 1000r

	Lethals from irradiated *SM1/+;Ubx^{130};+* males									
	Chromosome II					Chromosome III				
Days	4–6	6–8	8–10	10–12	Total	4–6	6–8	8–10	10–12	Total
Chromosomes	41	46	38	42	167	46	39	42	45	172
Lethals	7	11	9	5	32	7	9	8	5	29
Percentage	17.1	23.9	23.7	11.9	19.2	15.2	23.1	19.0	11.1	16.9
Inversions	..	..	1	..	1	..	..	1	1	2
Translocations	2	1	..	..	3	2	1	..	..	3

	Lethals from irradiated Canton isogenic males									
	Chromosome II					Chromosome III				
Days	4–6	6–8	8–10	10–12	Total	4–6	6–8	8–10	10–12	Total
Chromosomes	103	102	15	39	259	102	83	15	21	221
Lethals	5	8	1	1	15	13	5	1	0	19
Percentage	4.9	8.0	6.6	2.5	5.8	12.7	6.0	6.6	0.0	8.6
Inversions	..	1	..	..	1	..	..	..	..	0
Translocations	1	..	..	..	1	1	..	..	..	1
Difference in lethal percentage	12.2	15.9	17.1	9.4	13.4	2.5	17.1	12.4	11.1	8.3
Probability	0.05	0.02	0.32	0.24	0.001	0.40	0.02	0.45	0.25	0.03

genetic system. While hitherto there has been little direct evidence for such a phenomenon, asynapsis or the conflicting synaptic forces adjacent to inversion break points seem to account for this high level of mutability.

Extent of structural heterozygosity and mutation rate: In order to obtain lethals for a critical test of their non random linear distribution, the results of which are presented in a later section, males were also made heterozygous for single inversions in chromosome II, specifically the left-arm Curly inversion (*In 2L Cy*) or the pericentric Glazed (*Gla*) inversion. The use of less extensive structural heterozygosity permitted the incidental determination of whether previous mutability effects were unique properties of *SM1* and Ubx^{130} or whether other inversions might produce increases in mutability. Males heterozygous for the Curly or the Glazed inversions and Canton normal chromosomes were treated with 1000r of X-rays and mated in the manner described in the previous section, except that lethals in chromosome III were not scored.

The brood pattern of lethal mutation in these series is represented in Tables 6 and 7. While the levels of mutation are not as high as in irradiated *SM1*/+; Ubx^{130}/+ males, an increase over the Canton control is seen in five of the six post-spermatogonial broods. The intermediacy of these values is borne out in Figure 2, which represents the mutation rates obtained among the several irradiated sources. Although the mutation levels for *Cy*/+ and *Gla*/+ males show some similarity, the times of maximum lethal recovery are not the same. This disparity may reflect a difference in the rate of sperm maturation and discharge (STRØMNAES 1959).

The intermediacy in mutability of *Cy*/+ and *Gla*/+ males to *SM1*/+; Ubx^{130} and isogenic controls not only confirms the opinion that all the inversions tested

TABLE 6

Lethal frequency by brood: irradiated Gla/+ *series*

	Days			
	4–6	6–8	8–10	10–12
Chromosomes	77	121	74	43
Lethals	10	17	10	1
Percentage	13.0	14.0	13.5	2.4
Inversions	0	2	1	0

TABLE 7

Lethal frequency by brood: irradiated Cy/+ *series*

	Days			
	4–6	6–8	8–10	10–12
Chromosomes	82	60	57	42
Lethals	14	9	2	1
Percentage	17.1	15.0	3.5	2.4
Inversions	3	0	0	0

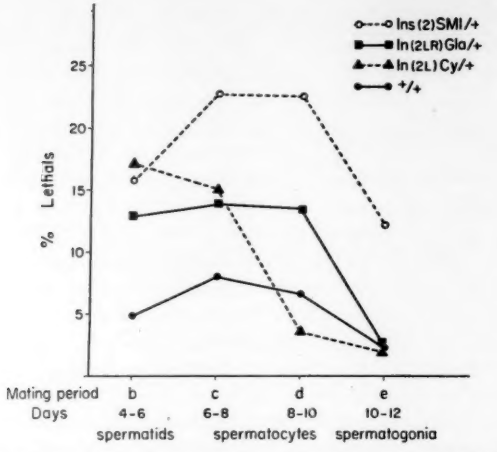

FIGURE 2.—The pattern of lethal mutations of chromosome II in irradiated lines.

increase mutation rates in meiotic stages but also indicates that the level of mutation may be a function of the degree of structural heterozygosity.

Distributions of lethals from heterozygous sources: It is clear from the discussion of the synergistic response of heterozygous lines to irradiation that, under the hypothesis of stress or mutability adjacent to existing break points, a second major consequence of localized asynapsis would be the preferential occurrence of both spontaneous and radiation-induced mutations at or near these points. As an additional test of the hypothesis, the lethals derived from each of the several heterozygous sources were located by their linkage relationships, and the distribution of mutated loci corresponding to each inversion was mapped.

Second chromosome lethals were located by their linkage with the dominant markers Sternopleural (*Sp*), Jammed (*J*), Lobe (*L*) and Pin (*Pin*). When a balanced stock of any new lethal had been obtained, males were mated to *Sp J L Pin/Gla* females, and the *Sp J L Pin*/lethal female progeny were tested for crossing over by mating them to *Gla*/lethal brothers. It may be seen that with free exchange between the lethal factor and the four markers, an array of non-Glazed progeny represents a direct test of crossing over. One of each of the reciprocal pairs of crossover and noncrossover classes will be eliminated as lethal. The nearer the lethal locus to a marker, the more extensively that marker predominates in the non-Glazed progeny. For example, if the lethal locus were at the same point as Lobe on the homologous chromosome, the non-Glazed offspring would be 100 percent Lobe. The map distance between the lethal and any marker may be estimated as the percentage of non-Glazed progeny without that marker.

In the same fashion chromosome-III lethals, which had been balanced with Ubx^{130}, were tested for their linkage with Roughened (*R*), Stubble (*Sb*), and Hairless (*H*).

In cases where initial tests with these markers indicated that the lethal being located was rather distant from all the markers used, other markers were used in supplementary crossover tests. These markers were Star (*S*) and Bristle (*Bl*) in chromosome II, and Glassy (*Gl*) and Prickle (*Pr*) in chromosome III. The use of all these markers gave close lethal location for both major autosomes. The only drawback to this method of lethal location is the variability in survival and expression of some mutant types. As an example, the accurate location of any lethal near Jammed had to be carried out at 28° C to ensure full expression of that mutant. In general, however, the internal consistency of the linkage data seemed to rule out severe difficulties of survival and classification.

Genetic map representations of the spontaneous lethal distributions derived from heterozygotes for *SM1* and Ubx^{130} are shown in Figure 3A. The estimated genetic position of each break point in the *SM1* and Ubx^{130} complexes is indicated by an arrow. Although there is a distinct clustering of lethals near the left break of *SM1*, and near the locus of the break left of the centromere in II, the pattern as a whole is unconvincing and is detracted from by the existence of breaks near the centromere, where a concentration of mutated loci would be expected in any case (DOBZHANSKY 1930, 1931).

The inversions *SM1* and Ubx^{130}, while they represent a very considerable departure from the homologies of a normally arranged chromosome and might contribute strongly to the observed increases in mutation frequency, are likely not to be as well suited to comparisons of the distribution of mutated loci. In several cases break points of the multiple inversions are quite close to each other. It seems possible that the change in homology is so extreme that certain extensive regions may not pair at all. The distributions associated with the Glazed and Curly inversions, which have widely separate break points in noncentric regions, present a much better opportunity to evaluate a clustering of lethal loci near the loci of breaks.

Genetic map representations of distributions obtained from the various X-rayed heterozygous sources are shown in Figure 3B. Figure 3C shows the distributions from the X-rayed Canton control. In these it was possible to designate the mating period during which each lethal was recovered, and the letters b, c, d, and e indicate recovery of a lethal 4–6, 6–8, 8–10, or 10–12 days after treatment, respectively. In this way the distributions of lethals can be correlated with the stages of spermatogenesis indicated in the frequency curves of Figure 2.

As a means of expressing quantitatively the degree of clustering of lethal loci near the genetic positions of inversion break points, a determination was made among the lethals from each heterozygous source of the fraction within three, five, and ten crossover units of the inversion breaks involved in that source. A similar determination was made among the control lethals from lines not having any structural heterozygosity. These values are compared in Table 8. In every case the fraction lying within five and ten crossover units of the critical loci was greater among lethals from the structurally heterozygous source than among those from the isogenic source. It is only when the limits of proximity are quite narrow (three crossover units) that in two cases out of six the control

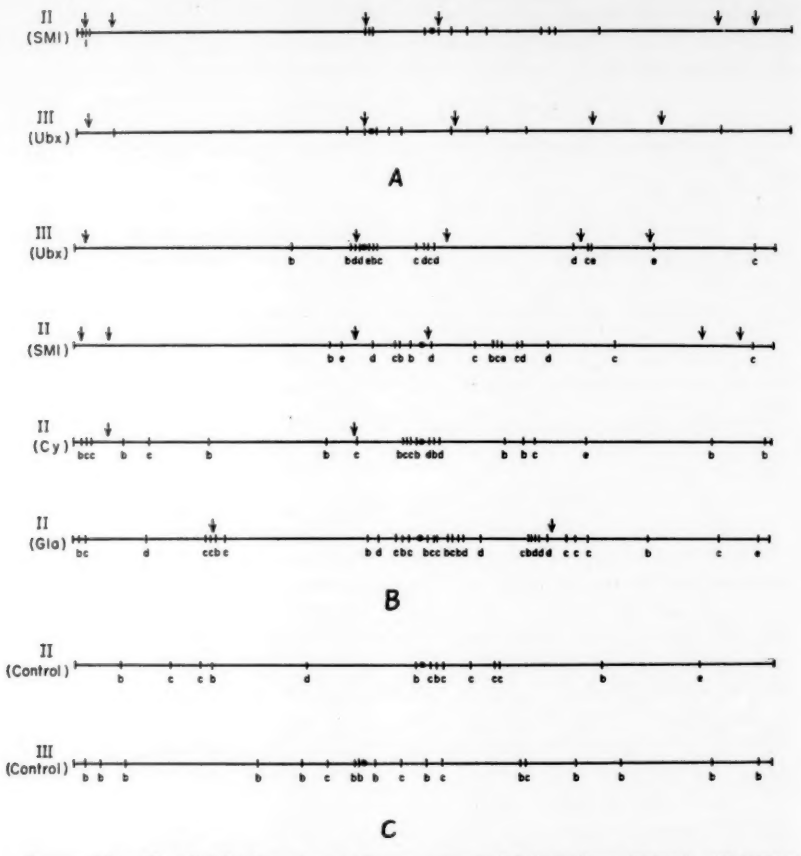

FIGURE 3A.—The distribution of spontaneous autosomal lethals from *SM1*/+; *Ubx*^{130}/+ parents. FIGURE 3B.—Distributions of autosomal lethals from structurally heterozygous males after irradiation. Letters indicate brood period of lethal recovery (see text and Figure 2). FIGURE 3C.—The distribution of autosomal lethals from isogenic Canton males after irradiation.

fraction is greater, and this fact may only demonstrate the diffuseness of lethal clusters in *SM1* and *Ubx*^{130} which was suggested earlier. Certainly in the case of lethals associated with the single inversions *Gla* and *Cy*, the clustering effect is most striking.

Thus, in the most favorably designed tests, the second expectation based on the hypothesis of stress or increased mutability adjacent to inversion breaks in structural heterozygotes is fulfilled. There appears to be a preferential localization of new mutations near existing break point loci when irradiation is combined with structural heterozygosity. This evidence, combined with the observations on fre-

TABLE 8

Clustering of lethals from heterozygous sources

Source of heterozygosity	Interval from break point loci					
	Three crossover units		Five crossover units		Ten crossover units	
	Experimental fraction	Control fraction	Experimental fraction	Control fraction	Experimental fraction	Control fraction
Spontaneous						
SM1/+	0.63	0.36	0.69	0.43	0.75	0.57
Ubx^{130}/+	0.40	0.44	0.60	0.56	0.90	0.72
Irradiated						
Ubx^{130}/+	0.69	0.44	0.88	0.56	0.88	0.72
SM1/+	0.31	0.36	0.44	0.43	0.63	0.57
Cy/+	0.14	0.07	0.29	0.07	0.52	0.21
Gla/+	0.26	0.14	0.35	0.14	0.39	0.14

quency of mutation, indicates that in one or both of the ways postulated the asynapsis concomitant with inversion loop formation results in an increase in local mutability.

A final point of evidence consistent with this conclusion must be made. Any new lethal mutations associated with inversion heterozygosity necessarily represent only a portion of the total number of lethals recovered, it being impossible to eliminate the residue of spontaneous or radiation-induced mutations for which structural heterozygosity is in no way responsible. Thus the distributions of Figure 3 represent an admixture of spontaneous and induced lethals, perhaps involving a great variety of underlying mechanisms of mutation. It is inescapable, however, that a fair number of these may be traced to some effect of inversion heterozygosity in meiotic stages as seen by the frequency increases in Figure 2. Scrutiny of the distributions in Figure 3B reveals that it is primarily lethals from the meiotic periods (c and d) which make up the most striking clusters adjacent to inversion breaks. This correlation of the time of greatest frequency increase and the time of strongest localization effect lends consistency to the interpretation that inversion breaks produce a marked local increase in mutability during some meitoic stage.

SUMMARY

1. The frequencies of spontaneous lethal mutation in normal chromosomes paired with the multiple-inversion chromosomes *SM1* and *Ubx*^{130}, and in identical normal chromosomes made homozygous, were determined. The rate of lethal mutation in inversion heterozygotes was significantly higher than in homozygous lines.

2. The increase in mutation associated with *SM1* and *Ubx*^{130} was found not to extend to the X chromsome.

3. Heterozygotes for *SM1* and *Ubx*^{130} were found to be so sensitive to X-ray treatment that a synergistic interaction of the genetic constitution and irradiation seems indicated.

4. The levels of mutation in X-rayed males heterozygous for the single inversions Curly and Glazed were found to be intermediate to the mutability of heterozygotes for the multiple inversions and that of isogenic controls during meiotic stages.

5. Lethals from all inversion-heterozygous sources were located by linkage tests, and the genetic map distribution of lethals from each source was determined. Evidence of the clustering of lethals near genetic loci corresponding to the inversion break points were found. This evidence was particularly strong in the distributions from single-inversion sources.

6. The accumulated evidence indicates an increase in mutability adjacent to the points of change in homology in inversion heterozygotes. Some alternative hypotheses toward an underlying mechanism are offered and evaluated.

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LITERATURE CITED

AUERBACH, C., 1941 The effect of sex on the spontaneous mutation rate in *Drosophila melanogaster*. J. Genet. **41**: 255–265.

1954 Sensitivity of the Drosophila testis to the mutagenic action of X-rays. Z. Ind. Abst. Vererb. **86**: 113–125.

BEADLE, G. W., 1932 A gene for sticky chromosomes in *Zea mays*. Z. Ind. Abst. Vererb. **63**: 195–217.

BRINK, R. A., 1958 Basis of a genetic change which invariably occurs in certain maize heterozygotes. Science **127**: 1182–1183.

CASPARI, E., and C. STERN, 1948 The influence of chronic irradiation with gamma-rays at low dosages on the mutation rate in *Drosophila melanogaster*. Genetics **33**: 75–95.

COCHRAN, W. G., 1942 The chi-square correction for continuity. Iowa State Coll. J. Sci. **15**: 421–436.

DOBZHANSKY, TH., 1930 Cytological map of the second chromosome of *Drosophila melanogaster*. Genetics **15**: 347–399.

1931 The decrease in crossing over observed in translocations and its probable explanation. Am. Naturalist **65**: 214–232.

DOBZHANSKY, TH., and C. EPLING., 1944 *Contributions to the General Taxonomy and Ecology of Drosophila pseudoobscura and Its Relatives*. Carnegie Inst. Wash. Publ. **554**.

GOLDSCHMIDT, R. B., 1955 *Theoretical Genetics*. Univ. of Calif. Press. Berkeley.

IVES, P. T., 1950 The importance of mutation rate genes in evolution. Evolution **4**: 236–252.

LEWIS, E. B., 1952 Report of new mutant. Drosophila Inform. Serv. **26**: 66.

LEWIS, E. B., and R. MISLOVE, 1953 Report of new mutant. Drosophila Inform. Serv. **27**: 57–58.

LI, C. R., 1957 *Introduction to Statistical Inference*. Edwards. Ann Arbor.

MCCLINTOCK, B., 1951 Chromosome organization and genic expression. Cold Spring Harbor Symp. Quant. Biol. **16**: 13–48.

MAMPELL, K., 1945 Analysis of a mutator. Genetics **30**: 496–505.

MULLER, H. J., 1954 The manner of production of mutations by radiation. Ch. 8, pp. 475–626. *Radiation Biology*, Vol. 1, Pt. 1. Edited by A. HOLLAENDER. McGraw-Hill. New York.

NOVITSKI, E., 1946 Chromosome variation in *Drosophila athabasca*. Genetics **31**: 508–524.

RHOADES, M. M., 1941 The genetic control of mutability in maize. Cold Spring Harbor Symp. Quant. Biol. **9**: 138–144.

SANDLER, L., Y. HIRAIZUMI, and I. SANDLER, 1959 Meiotic drive in natural populations of *Drosophila melanogaster*. I. The cytogenetic basis of segregation-distortion. Genetics **44**: 233–250.

SANDLER, L., and Y. HIRAIZUMI, 1959 Meiotic drive in natural populations of *Drosophila melanogaster*. II. Genetic variation at the Segregation-distortion locus. Proc. Natl. Acad. Sci. U.S. **45**: 1412–1422.

SPENCER, W. P., and C. STERN, 1948 Experiments to test the validity of the linear r dose/mutation frequency relation in Drosophila at low dosage. Genetics **33**: 43–74.

STROMNAES, Ø., 1959 Stock differences in X-ray mutational sensitivity pattern of *Drosophila melanogaster*. Hereditas **45**: 221–229.

STURTEVANT, A. H., 1939 High mutation frequency induced by hybridization. Proc. Natl. Acad. Sci. U.S. **25**: 308–310.

WELSHONS, W. J., and W. L. RUSSELL, 1957 The effect of X-rays on the Drosophila testis and a method for obtaining spermatogonial mutation rates. Proc. Natl. Acad. Sci. U.S. **43**: 608–613.

CONCERNING EQUILIBRIA OF HETEROTIC LETHALS IN RANDOM MATING POPULATIONS WITH PARTICULAR REFERENCE TO l(2)55i IN DROSOPHILA MELANOGASTER[1]

TERUMI MUKAI[2] AND ALLAN B. BURDICK

Department of Biological Sciences, Purdue University, Lafayette, Indiana

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THE introduction of lethal genes into a random mating population has, on the whole, a deleterious effect (that is, an adverse effect on the mean adaptive value of the population). However, if the lethal genes show heterotic effect, they are maintained, and if the zygotic viabilities and/or female fecundities of the lethal heterozygotes are large, the genes tend to increase population productivity within a certain area of gene frequencies and, therefore, have a role in the evolution of the population.

IVES (1945), WALLACE (1950) and PROUT (1954) have reported the spontaneous lethal mutation rate in the second chromosome of *Drosophila melanogaster*. Their estimates are 2.6×10^{-5}, 1.0×10^{-5}, and 1.13×10^{-5} lethal mutations per locus per generation, respectively. IVES (1945) and WALLACE (1950) also estimated the number of second chromosome loci which can mutate to lethals. The estimated numbers were 495 (IVES) and 400 (WALLACE).

STERN and his collaborators (1952) estimated the viability effect of sex-linked recessive lethals in heterozygous condition. Their results show that 27 out of 75 sex chromosome recessive lethals had some heterotic effect on zygotic viability.

It may be assumed that the lethal mutation rate is 1.13×10^{-5} per locus per generation, which is PROUT's (1954) estimate (used because it is intermediate among the three estimates) and that the number of loci which can mutate to lethal is 1000 in *Drosophila melanogaster*.

If one third of the lethals show heterosis (STERN, CARSON, KINST, NOVITSKI, and UPHOFF 1952), each fly in each generation has a probability of 0.0038 ($= 1.13 \times 10^{-5} \times 10^{3} \times \frac{1}{3}$) of having a new heterotic lethal mutation. Disregarding genetic drift, which might reduce the above probability estimate by one half, we find that heterotic lethals should accumulate in a population, but the evidence indicates that they do not. Rarely is a single lethal present in more than a trace frequency in a population.

In an attempt to explain this contradiction by ascribing the results to the effect of modifying mutations in the genetic background, we will extend the development of mathematical models, previously used by MUKAI and BURDICK (1959),

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[2] Present address: National Institute of Genetics, Mishima, Japan.

in this report. In addition, population productivity will be defined as a general concept and discussed with respect to lethal heterozygous populations.

MATHEMATICAL MODELS AND RESULTS

Definition of parameters

MUKAI and BURDICK (1959) separated relative viability of lethal heterozygotes into female fecundity (f), male mating ability (m), sperm competitive ability (s), and zygotic viability (v) and defined the following parameters:

Q_n = frequency of lethal heterozygotes (noted as Aa) in the nth generation, aa being lethal.

$1 - Q_n$ = frequency of AA individuals in the nth generation.

Q = frequency of lethal heterozygotes in the equilibrium population.

m = relative mating ability of Aa males, that of AA males being 1.

f = relative fecundity of Aa females, that of AA being 1.

v = relative zygotic viability (hatchability + larval competitive ability + length of larval period) of Aa individuals, that of AA being 1.

s = relative competitive ability of "a" sperms, that of "A" sperms being 1.

n = the number of generations.

In addition to the above notations, the following are added for the purpose of calculation.

P = relative population productivity.

Δm, (Δf; Δv; Δs) = increment of change per generation of mating ability (female fecundity, zygotic viability, sperm competitive ability) of lethal heterozygous individuals caused by the epistatic effect of modifying mutations in the genetic background.

c = relative frequency of individuals in which mutations changing the viability of lethal heterozygotes take place at a given locus in one generation.

Models

Lethal genes are of three types with respect to their macrogenetic behavior, that is, autosomal (A), Drosophila type sex chromosomal (XY-type) (B), or Bombyx and chicken type sex chromosomal (ZW-type) (C).

A. *Autosomal lethals:*

(1.) *Zygotic viability* (v):—The necessary and sufficient conditions for maintenance of a lethal gene were presented by MUKAI and BURDICK (1959). They showed that when a lethal gene has a heterotic effect expressed in zygotic viability, namely, $v > 1$, this lethal is maintained in the population. The zygotic array in the equilibrium population is as follows: $1/(2v - 1)$ of AA, $(2v - 2)/(2v - 1)$ of Aa, and none of aa.

If a mutation takes place in the genetic background modifying the viability of

the lethal heterozygote (Aa), the following array will be produced:

Genotype:	αAA	βAA	αAa	βAa	aa
Viability:	1	1	v	$v+\Delta v$	0
Frequency:	$\frac{(1-c)}{(2v-1)}$	$\frac{c}{(2v-1)}$	$\frac{(2v-2)(1-c)}{(2v-1)}$	$\frac{(2v-2)c}{(2v-1)}$	0

where α and β (used here and subsequently) denote the original and the mutational genetic background, respectively. If complete dominance of the new mutation is assumed, the zygotic array in the next generation will be as follows:

$$(AA) = 1 - Q_1 = \frac{v}{v + 2(v-1)\ [v(1-c)^2 + c(2-c)\ (v+\Delta v)]}$$

$$(Aa) = Q_1 = \frac{2(v-1)\ [v(1-c)^2 + c(2-c)\ (v+\Delta v)]}{v + 2(v-1)\ [v(1-c)^2 + c(2-c)\ (v+\Delta v)]}$$

The one generation change in frequency of lethal heterozygotes (ΔQ) is as follows:

$$\Delta Q = \frac{2(v-1)\ [v(1-c)^2 + c(2-c)\ (v+\Delta v)]}{v + 2(v-1)\ [v(1-c)^2 + c(2-c)\ (v+\Delta v)]} - \frac{2v-2}{2v-1}$$

$$= \frac{2(v-1)\ (2-c)\ \Delta v}{(2v-1)\ \{v + 2(v-1)\ [v(1-c)^2 + c(2-c)\ (v+\Delta v)]\}} \quad \ldots\ldots\ldots (1)$$

If an equal number of positive and negative mutations (each having the same magnitude of effect) takes place, then the absolute values of the numerator in Formula (1) are the same for a positive and a negative mutation. However, the denominator changes in accordance with the sign of Δv; the absolute value of ΔQ, therefore, is greater for a negative Δv than for a positive Δv. This means that a lethal gene, even though it shows superviability ($v > 1$) expressed in zygotic viability and is therefore capable of achieving equilibrium, will tend to be eliminated from a population as a result of random spontaneous mutational changes in the lethal-heterozygote-viability-modifying genetic background.

(2.) *Female fecundity* (f) [*or male mating ability* (m)]:—If female lethal heterozygotes have greater fecundities ($f > 1$) than wild-type females, they are maintained in the population (MUKAI and BURDICK 1959). The zygotic array in the equilibrium population is as follows: $R-1$ of AA, $2-R$ of Aa, and none of aa where

$$R = (1 + \sqrt{2(f-0.5)^2 + 0.5})/f$$

If a mutation takes place in the genetic background modifying female fecundity of lethal heterozygotes, the following array will be produced:

Genotype:	$\alpha AA + \beta AA$	αAa	βAa	aa
Fecundity:	1	f	$f+\Delta f$	0
Frequency:	$R-1$	$(2-R)\ (1-c)$	$(2-R)c$	0

After one random mating generation, the following zygotic array is produced:

$$(AA) = 1 - Q_1 = \frac{2R^2(R-1)^2 + (R^2-2)\ (2-R)c\,\Delta f}{2[R^2(R-1) + (2-R)\ (1+0.5R)\ (R^2-2)c\,\Delta f]}$$

$$(Aa) = Q_1 = \frac{(2-R)\ [R^2\ (R-1) + (R^2-2)\ c\,\Delta f]}{R^2(R-1) + (2-R)\ (1+0.5R)\ (R^2-2)\ c\,\Delta f}$$

The change in frequency of lethal heterozygotes (ΔQ) is as follows:

$$\Delta Q = \frac{(2-R)\,[R^2(R-1) + (R^2-2)\,c\,\Delta f]}{R^2(R-1) + (2-R)\,(1+0.5R)\,(R^2-2)\,c\,\Delta f} - (2-R)$$

$$= \frac{(2-R)\,(R^2-2)^2\,c\,\Delta f}{2[R^2(R-1) + (2-R)\,(1+0.5R)\,(R^2-2)\,c\,\Delta f]} \quad \ldots\ldots\ldots\ldots\ldots (2)$$

where, when $f > 1$

$$(2-R) = \frac{2(f-1)}{2f - 1 + \sqrt{2(f-0.5)^2 + 0.5}} > 0$$

$$\text{and } (R^2-2) = \frac{2}{\sqrt{2(f-0.5)^2 + 0.5} + (f-1)} > 0$$

And, under the same assumptions about mutation as in the case of zygotic viability, the same conclusion can be drawn from Formula (2), *viz.*, the lethal, even though capable of equilibrium, will tend to be eliminated.

In the case of superior mating abilities of males ($m > 1$), the same conclusion can be drawn, other things being equal, because m and f are interchangeable in the above formulae.

(3) *Sperm competitive ability* (s):—If the lethal-carrying sperms show better competitive abilities for fertilization than the lethal-free sperms, namely, $s > 1$, the lethal is maintained in the equilibrium population (MUKAI and BURDICK 1959). The equilibrium zygotic array is as follows: $1/\sqrt{s}$ of AA, $1 - (1/\sqrt{s})$ of Aa, and none of aa.

If a mutation modifying the competitive ability of lethal-carrying sperm takes place in the genetic background, the following array will be produced:

Genotype:	$\alpha AA + \beta AA$	αAa	βAa	aa
Frequency:	$1/\sqrt{s}$	$(1 - 1/\sqrt{s})(1-c)$	$(1 - 1/\sqrt{s})c$	0

After one random mating generation, the population shows the following zygotic array:

$$(AA) = 1 - Q_1$$

$$= \frac{(\sqrt{s}+1)\,\{\sqrt{s}\,(1+s)\,(1+\sqrt{s}) + [\sqrt{s}\,(1-c) + s + c]\Delta s\}}{s(1+\sqrt{s})^2\,(1+s) + [(1+s)\,(s-c) + 2\sqrt{s}(s+c)]\Delta s}$$

$$(Aa) = Q_1$$

$$= \frac{(\sqrt{s}-1)\,\{(1+s)\,\sqrt{s}(1+\sqrt{s})^2 + [2(s+c) + \sqrt{s}(1+s)]\Delta s\}}{s\,(1+\sqrt{s})^2(1+s) + [(1+s)(s-c) + 2\sqrt{s}(s+c)]\Delta s}$$

The change in frequency of lethal heterozygotes (ΔQ) is as follows:

$$\Delta Q = Q_1 - Q$$

$$= \frac{(\sqrt{s}-1)(s+1)\,c\,\Delta s}{s\sqrt{s}(1+s)(1+\sqrt{s})^2 + \sqrt{s}\,[(1+s)(s-c) + 2\sqrt{s}(s+c)]\Delta s} \quad \ldots\ldots (3)$$

Under the same assumptions about mutation as in the cases of zygotic viability, female fecundity (or male mating ability), we can draw a similar conclusion, *viz.*, the lethal, even though capable of equilibrium by virtue of $s > 1$, will tend to be eliminated.

B. *Drosophila-type sex chromosomal lethals (XY-type)*:

1. *Zygotic viability* (v):—It can be shown that, if sex-linked lethal heterozygous females show more than twice the zygotic viability of lethal-free females, namely, $v > 2$, the lethal will reach equilibrium and be maintained in the population. The zygotic array in the equilibrium population is as follows: $1/(v-1)$ of AA, $(v-2)/(v-1)$ of Aa, none of aa, and all males will be AY where Y stands for the Y chromosome.

If a mutation modifying the lethal heterozygote viability takes place in the genetic background, the following array will be produced:

Genotype:	αAA	βAA	αAa	βAa	αAY	βAY
Zygotic Viability:	1	1	v	$v+\Delta v$	1	1
Frequency:	$\frac{(1-c)}{(v-1)}$	$\frac{c}{(v-1)}$	$\frac{(v-2)(1-c)}{(v-1)}$	$\frac{(v-2)c}{(v-1)}$	$(1-c)$	c

After one generation of random mating, the following population would be obtained:

$$(AA) = 1 - Q_1 = \frac{v}{v(v-1) + (v-2)(2-c)\,c\,\Delta v}$$

$$(Aa) = Q_1 \quad = \frac{(v-2)\,[v + (2-c)\,c\,\Delta v]}{v(v-1) + (v-2)(2-c)\,c\,\Delta v}$$

$$\Delta Q = Q_1 - Q = \frac{(v-2)(2-c)\,c\,\Delta v}{(v-1)\,[v(v-1) + (v-2)(2-c)\,c\,\Delta v]} \quad \ldots\ldots\ldots\ldots (4)$$

The form of Formula (4) is the same as those given before, (1), (2), and (3), *viz.*,

$$\Delta Q = \frac{k_1 \Delta x}{k_2 + k_3 \Delta x}$$

where k_i is a positive function of the viability parameters (v, f, m or s) and the frequency of mutation (c), and Δx is the viability change magnitude associated with mutation in the genetic background. The lethal in this case, therefore, will tend also to be eliminated from the population.

2. *Female fecundity* (f):—The requirement for lethal equilibrium is $f > 2$. The equilibrium zygotic array is $f/[2(f-1)]$ of AA, $(f-2)/[2(f-1)]$ of Aa, none of aa, and all males AY. After a heterozygote-modifying mutation has taken place, the female array will be:

Genotype:	$\alpha AA + \beta AA$	αAa	βAa
Fecundity:	1	f	$f+\Delta f$
Frequency:	$\frac{f}{2(f-1)}$	$\frac{(f-2)(1-c)}{2(f-1)}$	$\frac{(f-2)c}{2(f-1)}$

One generation of random mating will produce

$$(AA) = 1 - Q_1 = \frac{f^2 + (f-2)\ c\,\Delta f}{2[f(f-1) + (f-2)\ c\,\Delta f]}$$

$$(Aa) = Q_1 = \frac{(f-2)(f + c\,\Delta f)}{2[f(f-1) + (f-2)\ c\,\Delta f]}$$

$$\Delta Q = Q_1 - Q = \frac{(f-2)\ c\,\Delta f}{2(f-1)\ [f(f-1) + (f-2)\ c\,\Delta f]} \quad \ldots\ldots\ldots\ldots (5)$$

Formula (5) has the same form as those shown previously.

The male viability parameters, m and s, are not involved since sex-linked lethals will not exist in males.

C. *Chicken-type sex chromosomal lethals (ZW-type)*:

The following general relationship of the pertinent parameters in the equilibrium population can be shown for the case where the lethal is sex-linked in a species with heterogametic females under the condition of $1 + s < smv$:

$$Q = \frac{1 + s - smv}{1 + s - smv - m} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots (6)$$

Formula (6) can be used to show that: (1) high values of s will not keep a lethal in a population, (2) m and v, while capable of equilibrium-generating values, are subject to the same type of ΔQ relationship as has been shown for f and v in the case of sex-linked lethals in the XY system.

All of the above formulae lead to the same conclusion with regard to the destiny of a recessive lethal in a random mating population: Random mutations in the lethal-heterozygote-viability-modifying background will tend to eliminate the lethal from the population even though the lethal heterozygote has a viability parameter sufficiently high to produce equilibrium. This will be true if the background mutations are equally likely to be positive or negative modifiers and if the absolute magnitude of the different mutations is the same.

EXPERIMENTAL RESULTS

MUKAI and BURDICK (1959) established four artificial autosomal lethal heterozygous populations involving the second chromosome recessive lethal *l(2)55i* in *Drosophila melanogaster*. In Population 1 which had high homozygosity in the genetic background, and in Population 2 which had high heterozygosity in the starting generation, both of which started with a frequency of lethal heterozygotes, $Q_1 = 1$, lethal heterozygotes reached equilibrium in the seventh generation with average frequencies of $Q = 0.42$. In Population 3 which had genetic background derived from Population 1, and in Population 4 with the genetic background of Population 2, the frequencies of the lethal started at $Q_1 = 0.05$ and increased to about the same values obtained for the first two populations. After 30–60 generations, SCHNICK, MUKAI, and BURDICK (1960) again estimated the frequencies of lethal heterozygotes in the above mentioned artificial populations.

The combined results of these two papers are recalculated and summarized in Table 1 and graphically presented in Figure 1 ($Q_n = \frac{2}{1+n}$ is the expected frequency if the viability of $l/+$ is the same as $+/+$). The results in the bottles of each generation are pooled for the sake of convenience, although there are heterogeneities between subpopulations within generations in Population 2 (generation 46–50) and Population 4 (generation 27–31). Populations 2 and 4 consist of seven and ten subpopulations, respectively, cultured in different milk bottles.

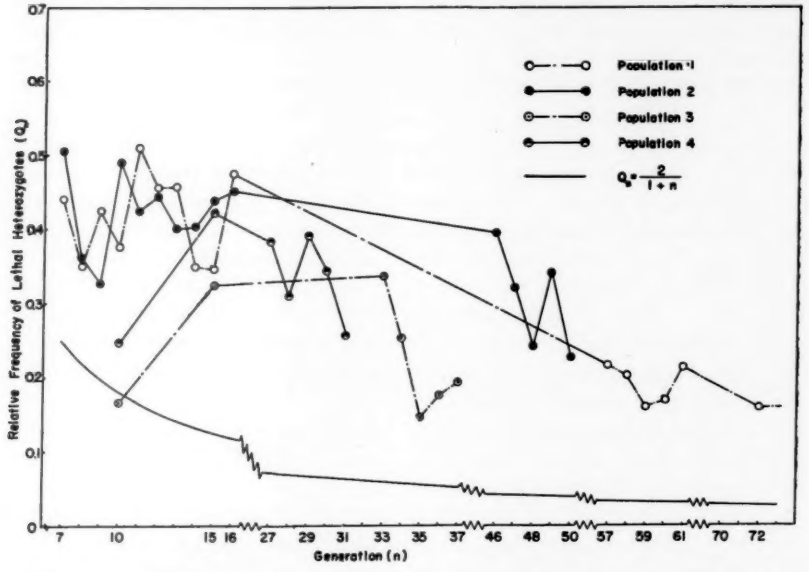

FIGURE 1.—Frequency of *l(2)55i* heterozygotes in various populations compared with the expected frequency, $Q_n = 2/[1+n]$.

TABLE 1

Temporary equilibrium frequencies in various l(2)55i *heterozygous populations*

Generation	Population 1	Population 2	Population 3	Population 4
Q_7–Q_{16}	0.420±0.019	0.424±0.017	0.326±0.032*	0.423±0.030*
Q_{27}–Q_{31}	.....	.....	.....	0.337±0.025
Q_{33}–Q_{37}	.....	.....	0.220±0.034	.....
Q_{46}–Q_{50}	.....	0.305±0.031	.....	.....
Q_{57}–Q_{61}	0.193±0.011	.....	.....	.....
Q_{72}	0.159±0.055†	.....	.....	.....

* Frequency estimated in the 15th generation only.
† This was estimated from a population which had been held in a large cage, and which had been established from generation 64 of population 1. The generation, 72, is an estimate based upon a 15-day life cycle.

In each population the frequencies of lethal heterozygotes have decreased significantly with generations. The temporary equilibrium values from the seventh to the 16th generation are 0.420 and 0.424 in Populations 1 and 2, respectively. The frequency of lethal heterozygotes dropped to 0.193 (generation 57–61), to 0.159 (estimated generation 72) in Population 1 and to 0.305 (generation 46–50) in Population 2. Although there are different genetic backgrounds in Populations 1 and 2, they are comparable because they have almost the same temporary equilibrium frequency during the period generation 7–16. The frequency of lethal heterozygotes from the 57th to 61st generation ($Q = 0.193$) in Population 1 is significantly smaller than that from the 46th to the 50th generation ($Q = 0.305$) in Population 2 [$\chi^2_{\text{d.f.}=1} = 14.11$ ($p < 0.0005$) by the 2×2 contingency table method].

In the early generations of Populations 3 and 4, only the frequencies in generations 10 and 15 were estimated. The result in the 15th generation, therefore, is not a good estimate of the temporary equilibrium frequency in these populations if the effect of genetic drift is taken into consideration. However, these are the best estimates we have for the early generations of these populations. The mean frequencies of lethal heterozygotes from generation 33 to generation 37 in Population 3, and those from generation 27 to generation 31 in Population 4 dropped significantly. In Population 3, $\chi^2_{\text{d.f.}=1} = 9.13$ ($0.005 > p > 0.001$). In Population 4, $\chi^2_{\text{d.f.}=1} = 6.37$ ($0.025 > p > 0.01$). It should be stressed that the results of Population 4 show a smaller χ^2 value than those of Population 3, probably because the frequencies of lethal heterozygotes in Population 4 were estimated in earlier generations (generation 27–30).

The demonstrated gradual decline of lethal frequency in these four populations is to be expected on the basis of the lethal-heterozygote-viability-modifying mutation hypothesis, previously described.

Lethal genes and population productivity

In general, population size will be relatively stable in a closed environment. A population containing a heterotic lethal will have the lethal at equilibrium frequency, although the reproductive potential of the population might not be at the maximum value. If such a population should suddenly expand to infinite size, the reproductive potential would govern the rate at which it would expand. We have chosen to call the reproductive potential, population productivity (P), and to define it as the potential offspring of the population. The relative ability of different populations to evolve, or expand, is partly a function of P, and therefore P is a component of the adaptive value of a population. Obviously the value of P is a function of gene frequency and the magnitude of the lethal heterozygote viability parameter. In the following models we consider that P is unity in the homozygous wild-type population and that only one viability parameter at a time is varying.

A. *Autosomal lethals:*

(1.) *Zygotic viability* (v):—In the case where v varies, population productivity can be expressed as:

$$P = \frac{1}{4}\left\{(1-2v)\left[Q - \frac{2(v-1)}{2v-1}\right]^2 + \frac{4v^2}{2v-1}\right\} \quad \ldots\ldots\ldots (7)$$

This formula indicates that P is maximum when $Q = \frac{2(v-1)}{2v-1}$ and that P will be greater than one at certain values of Q if v is greater than one. $Q = \frac{2(v-1)}{2v-1}$ also happens to be the equilibrium frequency of lethal heterozygotes where $v > 1$ (the minimum for maintenance of a lethal in a population) which means that population productivity will be at a maximum when the population is in equilibrium. The relationships between Q, v, and P are graphically shown in Figure 2.

(2.) *Female fecundity* (f):—Where f varies, population productivity is

$$P = 1 + \frac{(f-1)^2}{f} - \frac{f}{4}\left[Q - \frac{2(f-1)}{f}\right]^2 \quad \ldots\ldots\ldots\ldots (8)$$

and P maximum is obtained when $Q = \frac{2(f-1)}{f}$ which is always larger than $Q = 2 - [1 + \sqrt{2(f-0.5)^2 + 0.5}]/f$, the equilibrium frequency of lethal heterozygotes. Figure 3 shows P as a function of f and Q.

(3.) *Male mating ability* (m):—Population productivity is

$$P = 1 - \frac{mQ^2}{4(1-Q+mQ)} \quad \ldots\ldots\ldots\ldots\ldots\ldots (9)$$

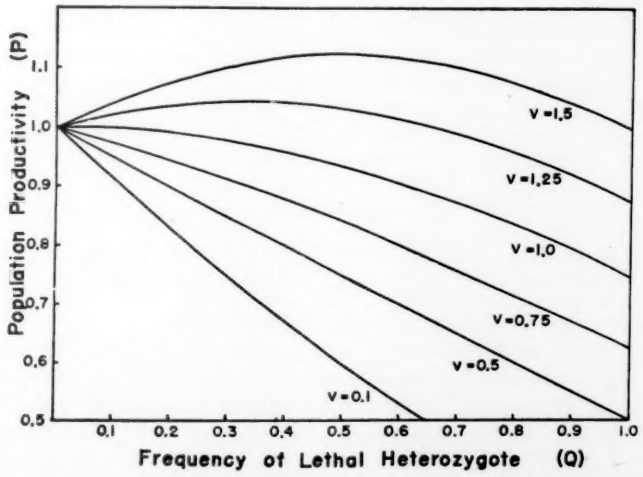

FIGURE 2.—Relationship between P and Q for autosomal lethals with various values of v.

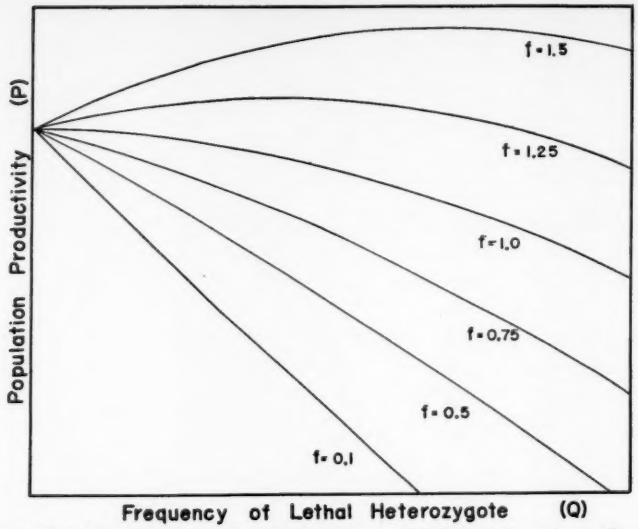

FIGURE 3.—Relationship between P and Q for autosomal lethals with various values of f.

We can see that,

$$\frac{mQ^2}{4(1-Q+mQ)} > 0$$

and, therefore, high m values of lethal heterozygotes would not increase population productivity.

(4.) *Sperm competitive ability* (s):—If l-carrying and wild-type sperm are considered to have varying competitive abilities in effecting fertilization, population productivity can be expressed as follows:

$$P = 1 - \frac{sQ}{2(1+s)} \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (10)$$

Since

$$\frac{sQ}{2(1+s)} > 0$$

we can conclude that l-carrying sperm always decrease population productivity whether or not they have greater-than-one competitive ability.

B. *Sex chromosomal lethals, Drosophila type* (XY):

(1.) *Zygotic viability* (v):—We consider that a sex-linked lethal will not exist in males under the XY sex chromosome system. Therefore, zygotic viability differences between lethal heterozygotes and homozygous wild types will be found only in females; homozygous wild-type males and females have equal viability. Population productivity in terms of v can be expressed as

$$P = 1 - \frac{Q(2-v)}{4} \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (11)$$

When v is larger than 2, P will be greater than one; as v increases above 2, P increases monotonically which is a contrast to the maximum for P (function of v) for autosomal lethals. The minimum v for maintenance of a sex-linked lethal in a population is also 2.

(2.) *Female fecundity* (f):—When f varies, population productivity can be expressed as

$$P = 1 - \frac{(4 - 3f)Q}{4} \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (12)$$

which indicates that P becomes larger than one, increasing monotonically, compared with the wild-type population, when $f > 4/3$.

Male mating ability and sperm competitive ability are not involved in sex-linked lethal viability since such lethals will not exist in males under the XY sex chromosome system.

C. *Sex chromosomal lethals, chicken type (ZW):*

(1.) *Zygotic viability* (v):—Population productivity in terms of v under the ZW sex chromosome system is exactly the same as under the XY system [Formula (11)].

(2.) *Female fecundity* (f):—Female fecundity is not considered because it is assumed that females will not carry lethals under the ZW system.

(3.) *Male mating ability* (m):—When m varies, P becomes

$$P = 1 - \frac{mQ^2}{4(1 - Q + mQ)}$$

which is exactly the same as Formula (9). It is clear that high values of m cannot increase population productivity.

(4.) *Sperm competitive ability* (s):—Population productivity in terms of s under the ZW sex chromosome system can be expressed by the same formula, (10), as that derived for s for autosomal lethals. The same conclusion, that lethal heterozygote heterosis cannot work through s to increase population productivity, is appropriate.

DISCUSSION

We have shown experimentally and theoretically that heterozygotes of *l(2)55i* are heterotic and capable of attaining equilibrium in two different genetic backgrounds (MUKAI and BURDICK 1959) and that the superviability of these heterozygotes is ultimately attributable to higher female fecundity *(f)* (SCHNICK, MUKAI and BURDICK 1960). In the present paper we are concerned about the effect of random mutational changes in the lethal-heterozygote-viability-modifying genetic background on equilibrium levels of superviable lethals. We find that random mutations in the background (each mutation with the same absolute magnitude of effect and equally likely to be a positive or a negative modifier) will tend to eliminate the lethal from the population even though the heterozygote has a viability parameter (m, v, f, or s) sufficiently high to produce stable equi-

librium. This expectation is born out in our later generation data on *l(2)55i* in which the frequency has been noted to decline from the original, apparently stable, equilibrium levels. Since all of the lethal situations (i.e., autosomal, sex-linked XY, sex-linked ZW) and each of the viability parameters give the same expectation in our theoretical examination, we are led to expect that no lethal will continue to exist indefinitely in a population unless new modifying mutations are continually more favorable than unfavorable for heterozygote viability (inversion and balanced lethal systems would maintain a lethal but we have extended our examination only to systems in which normal recombination takes place).

We have examined the question of whether lethals can be "adaptive" and contribute to the over-all fitness of a population. We find that under some circumstances, notably the one obtaining for *l(2)55i*, they are adaptive and favored by natural selection. The relative potential offspring of a population has been called population productivity (P). We have shown that, within a certain range of gene frequency, heterotic lethals increase P if they operate through the viability parameters v or f for autosomal or XY system sex-linked lethals or through v for ZW system sex-linked lethals. Heterotic lethals that operate through m or s, although capable of equilibrium, can only serve to reduce P, and the amount of reduction in P is related to the degree of heterosis.

For autosomal lethals, v or f need only be greater than unity to produce equilibrium but, for sex-linked lethals of either system, v must be greater than two, and f, which can only operate in the XY system, must be also greater than two to produce equilibrium. So, if heterotic lethals are found in populations, it is to be expected that they would be autosomal and operate through v or f. These considerations indicate the essential differences in the genetic systems governing autosomal and sex-linked lethals and are adequate reason for not extending generalizations from one type of lethal to another.

Random mating populations such as *Drosophila melanogaster* go through cycles of expansion and contraction where P is important. Therefore, if we find heterotic lethals in Drosophila, it seems more likely that they would operate through v or f since such lethals, for a given amount of associated heterosis, have a greater amount of reproductive potential under conditions of oscillating population size than those that operate through m or s. Our second chromosome lethal, *l(2)55i*, has been shown to operate at first probably through m and f and later only through f so that its existence in a wild population and its mode of action are to be anticipated from theoretical considerations.

The equilibrium level of lethal heterozygote frequency of an autosomal heterotic lethal that operates through f has been shown to be less than the level that produces maximum population productivity. This means that, during periods of population expansion, the lethal frequency might increase.

Our particular lethal, *l(2)55i*, is beneficial to individuals that carry it and to populations that contain it; other lethals of similar action might be found. But, the specifications for such lethals restrict them largely to the autosomes and call for action through v or f. Not every lethal is even potentially beneficial, and those

that are must act in only a certain way. On the other hand, this denies that all lethals are potentially harmful to populations and requires that the v and f values be known before the lethal effect can be assessed.

Finally, it must be said that even though a lethal is both capable of equilibrium and effective in increasing population productivity, ultimately it probably will be lost from the population as a result of "mutational static" in the lethal-heterozygote-viability-modifying background. The "beneficial lethal" effect of any given lethal, therefore, is likely to be transitory—the lethal might occur as a mutation, increase to its equilibrium level improving population productivity as it increases, and then slowly decline. The population might be better off for having had such a lethal, but, if so, the effect was that of a temporary stimulant. The frequency of this kind of lethal mutation is unknown—one has definitely been found in a wild population and described in this series of papers. But, if the frequency is even a small part of the total lethal mutation burden, the "beneficial lethal" effect might be a repeated experience of the population and an important attribute of its evolution.

SUMMARY

Random mutations tend to eliminate *l(2)55i* from a population even though the heterozygote has a viability parameter sufficiently high to produce stable equilibrium.

Theoretical considerations regarding other autosomal and sex-linked lethals are given.

LITERATURE CITED

IVES, P. T., 1945 The genetic structure of American populations of *Drosophila melanogaster*. Genetics **30**: 167–196.

MUKAI, TERUMI, and ALLAN B. BURDICK, 1959 Single gene heterosis associated with a second chromosome recessive lethal in *Drosophila melanogaster*. Genetics **44**: 211–232.

PROUT, T., 1954 Genetic drift in irradiated experimental populations of *Drosophila melanogaster*. Genetics **39**: 529–545.

SCHNICK, S. M., TERUMI MUKAI, and ALLAN B. BURDICK, 1960 Heterozygote viability of a second chromosome recessive lethal in *Drosophila melanogaster*. Genetics **45**: 315–329.

STERN, C., G. CARSON, M. KINST, E. NOVITSKI, and D. UPHOFF, 1952 The viability of heterozygotes for lethals. Genetics **37**: 413–449.

WALLACE, B., 1950 Allelism of second chromosome lethals in *Drosophila melanogaster*. Proc. Natl. Acad. Sci. U.S. **36**: 654–657.

BLOOD GROUPS IN SHEEP. III. THE A, C, D AND M SYSTEMS[1]

BENJAMIN A. RASMUSEN,[2] CLYDE STORMONT AND YOSHIKO SUZUKI

The School of Veterinary Medicine, University of California, Davis, California

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IN the course of studying the inheritance of phenogroups in the X-Z and B systems of blood groups in sheep (RASMUSEN 1958, 1960) it became apparent that some of the reagents used for blood typing of sheep were reacting in neither of these two systems nor in the R-O system. This report presents evidence for four additional systems of ovine blood groups detected by these reagents.

MATERIALS AND METHODS

The blood samples and the techniques used in performing the blood-typing tests and absorptions in this study were the same as those used for the studies of the X-Z and B systems (RASMUSEN 1958, 1960). Both isoimmune and heteroimmune antisera were used as sources of reagents.

Preparation of reagents

The A reagent: The A reagent (Table 1) was an unabsorbed, isoimmune antiserum. It was produced in a sheep named A, which received five intrajugular injections, at five-day intervals, of 5 ml of citrated blood from a sheep named B. The antiserum was collected five days after the last injection. Sheep A was of blood-group R in the R-O system, and a preimmunization sample of her serum contained neither natural hemolysins nor agglutinins for the red cells of other sheep. Immune hemolysins were first demonstrated in the sample of serum collected just prior to the fifth or last injection. The antiserum, coded S3, was collected five days later.

This antiserum had a uniform titer of 1/128 in tests with the reactive bloods of different sheep, suggesting that it might contain a single, nonfractionable population of antibodies in tests with the red cells of sheep. Numerous absorptions were performed in which bloods from individual sheep were utilized, and in each instance the reactive bloods exhausted all of the hemolysins. The S3 antiserum was used routinely at a dilution of ¼ as the A reagent of this study. At this dilution regular and complete hemolysis was usually effected within one and one half hours after the tests were set up. A rare exception to the observation of complete hemolysis with this reagent occurred in tests of the red cells of a pair of twins, and in that instance it was possible to show that the incomplete hemo-

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[2] Present address: Department of Animal Science, University of Illinois, Urbana.

TABLE 1

Reactions of the erythrocytes of 15 rams with reagents for the A, C, D, M, R-O and X-Z systems

Red cells of rams		Reagents and antiserum numbers*											
		A S 3	C S 66	C C 58	C_x S 33	D S 21	M S 67	M_x S 57	M_x RTG 18	R C 86	O C 45	X RS 9	Z RS 19
Shropshire	114	+	+	+	+	0	+	+	+	+	0	+	0
Shropshire	30	+	+	+	+	0	+	+	+	+	0	+	0
Suffolk	669-0	0	0	0	+	0	+	+	+	+	0	+	0
Suffolk	76650	0	0	0	+	0	+	+	+	0	+	+	0
Suffolk	105335	0	0	0	+	0	±	+	+	+	0	+	0
Grade Suffolk	21	+	+	+	+	0	0	0	0	+	0	+	0
Corriedale	412	0	0	0	±	0	0	0	0	+	0	+	0
Targhee	5020T	+	+	+	+	+	0	0	0	+	0	+	+
Targhee	53035T	0	0	0	+	0	0	0	0	0	+	+	0
Targhee	9501T	+	0	0	+	+	0	0	0	+	0	+	+
Rambouillet	4458RW	0	0	0	+	0	0	0	0	0	+	+	0
Rambouillet	2516XW	+	0	0	+	0	0	0	0	0	+	+	+
Rambouillet	4375RW	+	0	0	+	+	0	0	0	+	0	+	0
Rambouillet	2435XW	+	0	0	+	0	0	+	+	+	0	+	+
Rambouillet	396XW	+	+	+	+	+	+	+	+	0	+	+	0

* Reagents C (S66), C_x (S33), M (S67), and M_x (S57), prepared from ovine isoimmune antisera and reagent C (C58) prepared from a bovine isoimmune antiserum, were tested using rabbit complement (absorbed at 4°C with sheep red cells). Reagent A (S3), prepared from a sheep isoimmune antiserum and reagent M_x (RTG18) prepared from a rabbit anti-Tahr-goat antiserum, were tested using guinea pig complement. The D reagent was used in agglutination tests. For tests involving the X and Z reagents see RASMUSEN (1958).

lysis was a result of erythrocyte mosaicism (STORMONT, WEIR and LANE 1953).

Sheep A was given a booster of 25 ml of the blood of sheep B approximately three months after antiserum S3 was collected, and eight days later a second antiserum was collected. This antiserum, coded S9, paralleled the S3 antiserum in titer and degree of reaction and reacted with the same erythrocytes as S3. All immunizations of A-negative sheep with the blood of A-positive sheep (seven trials) resulted in the production of A antibodies. Consequently, in subsequent series the immunization of A-negative sheep with A-positive blood was avoided.

The C and C_x reagents: The C reagent (S66, Table 1) was obtained from an antiserum prepared by injecting 10 ml of washed citrated blood from sheep 2083 into the jugular vein of sheep 575 (group O in the R-O system) twice a week for two weeks. This antiserum was collected four days after the last injection. Following absorption of S66 with human erythrocytes of group A to remove natural antisheep R, it behaved as if it contained a single, residual population of antibodies. The reagent was used at a dilution of ¼ in the present studies. Antisera S20, S24, S27, S37 and S68 provided other sources of C reagents. Another C reagent (C58, Table 1), was prepared from a bovine isoimmune antiserum. An additional C reagent was obtained from an antiserum produced by two injections, one week apart, of 10 ml of pooled washed erythrocytes from black-tailed deer (*Odocoileus hemionus columbianus*) into the jugular vein of sheep 658. This

antiserum, SBT1, was collected seven days after the last injection. It was not yet available when the bloods of the rams listed in Table 1 were tested.

The reagent named C_x was an antiserum, S33, produced in sheep 469604. This sheep received two 10 ml injections, eight days apart, of erythrocytes of sheep 311, followed by a 10 ml booster of the pooled bloods of sheep 999, 1000 and 1318 approximately one and one half years later. The antiserum was collected one week after the booster injection. Trial absorptions yielded no evidence for more than a single population of antibodies. A dilution of ¼ was chosen as optimal or near-optimal for C_x in the tests.

The D reagent: The D reagent (Table 1) was prepared by immunization of sheep 3539 (group O in the R-O system) with washed erythrocytes of sheep N772, N773, O831 and O832. Six injections, five days apart, of 10 ml of pooled erythrocytes were made, and the antiserum, S21, was collected one week after the last injection. It contained three distinct populations of antibodies against red cells of sheep: (1) natural anti-R; (2) a population called D which acted as agglutinating antibodies and which also caused some hemolysis in conjunction with rabbit complement but not with guinea pig complement; and (3) a population called E′ which reacted with certain phenogroups in the B system (RASMUSEN 1960) and produced hemolysis in conjunction with either rabbit or guinea pig complement.

This antiserum, after absorption with human red cells of group A to remove the natural anti-R, was used only with guinea pig complement and served as a D plus E′ reagent. Agglutination appeared much more rapidly after the tests were set up than did hemolysis, so that the reactions due to the D population of antibodies (observed as agglutination) were clearly distinguishable from those due to the E′ population (observed as hemolysis), inasmuch as guinea pig complement did not participate in the D antigen-antibody system. Erythrocytes reactive with both populations of antibodies (D and E′) were first agglutinated and then lysed. This report is concerned only with the reactions of the D population. These D antibodies were unique among the battery of reagents used for blood typing of sheep in these studies inasmuch as they were the only sheep blood-typing antibodies which reacted regularly as agglutinins. Reagents for all the other blood-group systems reacted as hemolysins (see footnote to Table 1 and also RASMUSEN 1958, 1960).

The M and M_z reagents: Three additional antisera were produced from reimmunizations of sheep 3539, and from each of these at least two reagents could be prepared. Fourteen months after the S21 antiserum was collected, sheep 3539 was given a single injection of 10 ml of pooled erythrocytes from sheep 999, 1000 and 2960. A second booster injection was made with cells of sheep 2355, and finally, a third booster injection, again with cells of sheep 2355, resulted in an antiserum, S57, which contained a population of antibodies not previously encountered. This new population was named M, and by appropriate absorption the M reagent was isolated. Another reagent, similar to the M reagent prepared from S57, was obtained from antiserum S67. This antiserum was produced by injection of 10 ml of washed erythrocytes of sheep 2083 into the jugular vein of sheep 652 twice a

week for three weeks and was collected four days after the last injection. S67 did not react with all of the bloods lysed by the original M reagent (S57), but the bloods lysed by S67 were always lysed by S57. After tests of several hundred blood samples were examined for comparison of the reactions of these two M reagents, the original M (S57) was designated M_x and that from S67 was designated M (Table 1).

A second source of M_x reagent was a rabbit antiserum, coded RTG18, produced by immunization with the red cells of Tahr goats (see STORMONT and SUZUKI 1958). The reagent was prepared by absorption of antiserum RTG18 with red cells from M-negative sheep.

EXPERIMENTAL RESULTS

Table 1 summarizes the reactions of the red cells of 15 rams with reagents for the A, C, D, M, R-O and X-Z systems. The rams headed the sire families which were used in the studies of the genetics of the blood-group systems. Their B phenogroups are given in Table 3 of the report on the B system (RASMUSEN 1960).

Inheritance studies

A study of the inheritance of the phenogroups reactive with the reagents A, C, C_x, D, M and M_x was made using the same bloods as those used in the studies of the X-Z and B systems (RASMUSEN 1958, 1960).

To determine whether any of these six reagents contained antibodies reactive with any of the numerous phenogroups in the B system, with phenogroups R and O of the R-O system, or with phenogroups X and Z of the X-Z system, their reactions with the red cells of lambs sired by rams known to be heterozygous for alleles at the B, R-O, and X-Z loci were examined. There was no evidence that any of the six reagents contained antibodies reactive with phenogroups in these three systems. On the contrary, the reactions of these six reagents with the erythrocytes of the lambs appeared to be completely independent of the reactions due to the B, R-O and X-Z phenogroups inherited from the rams. Consequently, the reactions of the reagents A, C, C_x, D, M and M_x must be ascribed to a system or systems other than B, R-O and X-Z.

It was thus necessary to determine the number of new systems that must be postulated to account for the reactions of the reagents A, C, C_x, D, M and M_x. If, for example, any two of these reagents, as A and C, are reacting in the same system, the phenogroup formulas with respect to those reactions must be either A/C or AC/− (− = no-A and no-C) in animals known to be heterozygous, i.e., animals with some offspring whose erythrocytes were not lysed by the reagent or reagents in question. For example, if a ram were of formula A/C, he would be expected to transmit A and C as alternatives. If he were of formula AC/−, he would be expected to transmit AC as an alternative to no-AC (−). If, however, the A and C reactions are due to phenogroups in different systems, then the appearance among the progeny of any three of the four possible types: (1) A and C; (2) A and no-C; (3) no-A and C; or (4) no-A and no-C; or all four of

these types, as transmitted by the ram alone, would establish that separate systems are involved in the reactions of A and C.

Data in Table 2 for 13 offspring of a Targhee ram, 5020T, show that the phenogroups A as contrasted with no-A (−), C as contrasted with no-C (−) and D as contrasted with no-D (−) are in three separate systems and are not in the B, R-O or X-Z systems. Data in Table 3 for six selected offspring of Shropshire ram 114 show that phenogroup M contrasts with no-M (−) in a system separate from the B, R-O, A and C systems. In Table 4, results are presented which show that the M_x reagent is not reacting with phenogroups in the X-Z system. While the data in Table 5 show that the M system is separate from the D system, they are

TABLE 2

Inheritance of B, R-O, X-Z, A, C and D phenogroups of ram 5020T (Targhee) by 13 of his offspring

Offspring number	Phenogroups from sire in blood-group system					
	B	R-O	X-Z	A	C	D
6	Q1†	*	X	*	—	*
11	Q1	*	X	—	—	D
14	Q1	O	Z	*	C	—
15	Q1	R	Z	*	—	D
19	Q1	R	Z	—	C	—
27	Q1	R	X	A	C	D
38	Q1	O	Z	*	C	—
39	Q1	R	X	*	—	*
49	Q1	O	X	*	C	*
67	Q1	O	*	—	—	D
33	I5‡	O	X	—	*	—
50	I5	O	*	*	C	*
66	I5	R	*	*	*	D

* Contribution of sire not determined.
† Q1 = $BB'ENO'_xQ$.
‡ I5 = $BB'E_xI_1Y$.

TABLE 3

Inheritance of B, R-O, A, C and M phenogroups of ram 114 (Shropshire) by six of his offspring

Offspring number	Phenogroups from sire in blood-group system				
	B	R-O	A	C	M
423	Q9†	R	A	—	M
428	*	*	A	—	—
431	*	O	*	C	—
464	Q9	O	—	—	—
475	Q'2‡	O	—	*	—
476	Q'2	O	—	C	M

* Contribution of sire not determir
† Q9 = O'_xQ.
‡ O'2 = $BB'EO'_1T_3U_1$.

TABLE 4

Inheritance of M and X-Z phenogroups of ram 2435XW (Rambouillet) by four of his offspring

Offspring number	Phenogroups from sire in blood-group system	
	M	X-Z
3917XW	M_x	Z
3918XW	M_x	X
3722XW	M_x	X
2048US	—	Z

TABLE 5

Inheritance of D and M phenogroups of ram 396XW (Rambouillet) by three of his offspring

Offspring number	Phenogroups from sire in blood-group system	
	D	M
3535XW	—	—
3724XW	—	M
3986XW	—	—

TABLE 6

Frequency of D and M in 98 white-faced ewes

	M	—	Total
D	5	56	61
..	4	33	37
Total	9	89	98
Chi-square = 0.18	d.f. = 1	$P > 0.05$	

TABLE 7

Frequency of D and M in 75 black-faced ewes

	M	—	Total
D	5	4	9
..	39	27	66
Total	44	31	75
Chi-square = 0.04	d.f. = 1	$P > 0.05$	

perhaps somewhat inconclusive due to lack of D-positive offspring of ram 396XW in matings to D-negative ewes. The two-by-two distributions shown in Tables 6 and 7 indicate that the D and M reactions must involve separate systems.

In Table 6 no attempt was made to distinguish between the subtypes M and M_x in the M system. In the C system, only phenogroups C and − (no-C) were distinguished in Tables 2 and 3. Actually, phenogroup C_x was not included in the analysis of sire families because of its high frequency and the consequent difficulty in finding matings of heterozygous rams with C_x-negative ewes for family

studies. It was placed in the C system primarily as a result of the following criteria: (1) Bloods that were lysed by the C reagent were invariably lysed by C_x, thereby indicating a relationship between C and C_x. (2) The cross-reactions of C_x with bovine erythrocytes were entirely limited to phenogroups in the C system of cattle just as were the cross-reactions of the C reagent C58. (S66 did not react with cattle blood). (3) The limited genetic data available were also in accord with the hypothesis that C_x was reacting in the C system.

Table 8 summarizes the blood groups of the ewes from the flocks studied, including 75 Shropshires, 23 Suffolks, 8 Corriedales, 29 Targhees and 71 Rambouillets (including some Rambouillet-Merino crossbreds).

The two phenotypes, A-positive and A-negative, in the A system were encountered in all breeds studied, and in no breed was the A-negative phenotype observed in more than half of the blood samples for that breed (Table 8).

The frequency of phenotypes C and C_x differed considerably between the Shropshires, which were largely of phenotype C, and the other four breeds, which were largely C_x. The frequency of C was the most striking difference between the two black-faced breeds (Shropshires and Suffolks) observed in these studies (Table 8).

In the D system, D-positive animals were rather infrequent in the black-faced breeds but were more frequent in white-faced breeds (Corriedales, Targhees and Rambouillets). Conversely, in the M system, the M-positive phenotypes were more frequent in the black-faced breeds (Table 8).

The data of Table 8 may not reflect accurately the frequencies of blood groups

TABLE 8

Phenotypes of ewes in six blood-group systems

System	Phenotype	Black-faced breeds		White-faced breeds		
		Shropshire	Suffolk	Corriedale	Targhee	Rambouillet
R-O	R	62	5	4	14	39
	O	13	17	4	12	39
	i*	0	1	0	3	0
A	A	49	15	4	21	58
	—	26	8	4	8	13
C	C	67	0	0	0	8
	C_x	2	22	8	29	61
	—	6	1	0	0	2
D	D	7	2	3	24	32
	—	68	21	5	5	39
M	M	43	18	0	4	5
	M_x	0	0	0	0	3
	—	32	5	8	25	63
X-Z	X	75	23	5	19	39
	XZ	0	0	2	10	29
	Z	0	0	1	0	3

* i=no-R, no-O.

for the entire breed for any of these blood-group systems, since only a few flocks were studied.

As is shown in Table 8, two phenotypes may be distinguished in the A system, three in the C system, two in the D system and three in the M system. If a small letter is used for the allele which when homozygous results in a negative blood type, the symbols for the alleles at each of the four loci may be written as follows (in descending order of dominance): (1.) *A, a.* (2.) *C, C^x, c.* (3.) *D, d.* (4.) *M, M^x, m.*

DISCUSSION

The C system of sheep appears to be homologous to the complex C system of cattle, on the basis of studies of cross-reactions of bovine and ovine isoimmune sera (STORMONT, SUZUKI and RASMUSEN 1957). In sheep our present knowledge of the C system is limited to the observation, by the use of two reagents, of only three phenotypes (C, C_x and no-C). Further studies with new reagents may reveal that this system in sheep is as complex as the C system of cattle (see STORMONT, OWEN and IRWIN 1951).

Of the other three systems (A, D and M) described in this paper, the M system appears to be the most complex. The reactions of the M-system reagents available clearly differentiated three phenotypes (M, M_x and no-M), and there was evidence which suggested that several more phenotypes exist, which for the present have been considered together simply as M. For example, one of the heteroimmune antisera produced in sheep by immunization with cattle blood contained antibodies which reacted almost exclusively with homozygotes for an unclassified subtype of M found in Shropshires.

Preliminary studies of cross-reactions of the M-system reagents indicate that the M system of sheep may be homologous to the S-U system of cattle, but the data are as yet inconclusive.

SUMMARY

Evidence is presented for four systems of ovine blood groups: A, C, D and M. These, in conjunction with B, R-O and X-Z, bring to seven the number of blood-group systems currently recognized in sheep.

Presently, there are two phenogroups in the A system, A as contrasted with no-A. Antibodies against phenogroup A are regularly engendered in A-negative sheep immunized with the blood of A-positive sheep. Isoimmune antisera have provided the sole source of A reagents.

Similarly, there are at present two phenogroups in the D system, D as contrasted with no-D. A single ovine isoimmune antiserum provided the source of D antibodies which acted typically as agglutinins in contrast with the reagents utilized in the studies of the other systems, which reacted typically as hemolysins.

Multiple alleles appear to be involved in the control of phenogroups in the C and M systems. Both ovine and bovine isoimmune antisera have provided sources of reagents reactive with phenogroups in the C system. Reagents reactive with phenogroups in the M system have been obtained primarily from ovine iso-

immune antisera, but certain M reagents were also prepared from heteroimmune antisera.

The C system of sheep appears to be homologous to the C system of cattle.

LITERATURE CITED

RASMUSEN. B. A., 1958 Blood groups in sheep. I. The X-Z system. Genetics **43**: 814–821.
1960 Blood groups in sheep. II. The B system. Genetics **45**: 1405–1417.

STORMONT, CLYDE, R. D. OWEN, and M. R. IRWIN, 1951 The B and C systems of bovine blood groups. Genetics **36**: 134–161.

STORMONT, CLYDE, and YOSHIKO SUZUKI, 1958 The distribution of Forssman blood factors in individuals of various Artiodactyl species. J. Immunol. **81**: 276–284.

STORMONT. CLYDE, YOSHIKO SUZUKI, and B. A. RASMUSEN, 1957 Cross-reactions of ovine and bovine isoimmune antibodies in cattle and sheep blood-typing studies. (Abstr.) J. Animal Sci. **16**: 1102.

STORMONT, CLYDE, W. C. WEIR, and L. L. LANE, 1953 Erythrocyte mosaicism in a pair of sheep twins. Science **118**: 695–696.

GENETICAL AND BIOCHEMICAL STUDIES OF HISTIDINE-REQUIRING MUTANTS OF NEUROSPORA CRASSA. I. CLASSIFICATION OF MUTANTS AND CHARACTERIZATION OF MUTANT GROUPS[1]

BROOKE B. WEBBER[2] AND MARY E. CASE

Department of Botany, Josiah Willard Gibbs Research Laboratories, Yale University, New Haven, Connecticut

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SEVEN histidine-requiring mutants of Neurospora were described by HAAS, MITCHELL, AMES and MITCHELL (1952) and placed in four distinct genetic groups (subsequently designated *hist-1* through *hist-4* by BARRATT, NEWMEYER, PERKINS, and GARNJOBST 1954).One of these groups *(hist-3)* was shown to comprise mutants of at least two distinct biochemical classes. MATHIESON and CATCHESIDE (1955) also reported studies of a *hist-2* mutant, K-12, a *hist-3* mutant, K-26, and a distinct new mutant, K-34.

The classification by heterocaryon tests of 704 additional histidine mutants which were derived in genetically closely related wild-type strains from three filtration-concentration experiments is described here. As the classification proceeded, new groups were found which had not been described previously in the literature. Representatives of the various groups were studied physiologically and genetically to characterize the groups with respect to their positions in the histidine biosynthetic scheme. The classification and characterization of various groups will be described here in detail.

MATERIALS AND METHODS

The 704 histidine-requiring mutants were obtained from three filtration-concentration experiments (Y152, Y155, and Y175) from untreated and X-irradiated macroconidia. These mutants are designated by experiment number (Y....) and arbitrary mutant number (M....). In these experiments three wild-type strains were used, namely 74A (from DR. PATRICIA ST. LAWRENCE) and two very closely related strains, 5.5A and 3.1a (CASE and GILES 1958). The X-ray dose (10,000r to 36,000r in different experiments) was administered at 4000r/minute with a General Electric Maxitron 250 unit operated at 250 kv and 30 milliamperes with a 1 mm aluminum filter. Other strains used in the genetic analyses are listed in Table 1.

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[2] Present address: Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee.

TABLE 1

Mutant strains used in genetic analyses

Isolation number	Locus designation(s)*	Phenotype
C84	*hist-1*	requires histidine
C94	*hist-2*	requires histidine
C140	*hist-3*	requires histidine
T1710	*hist-3*	requires histidine
C141	*hist-4*	requires histidine
K-26	*hist-3*	requires histidine
K-34		requires histidine
10575	*tryp-1*	requires tryptophane
74-YU938-1a	*arg-3 nic-2*	requires arginine and nicotinamide
74-OR14-6A	*ad-5 nic-2*	requires adenine and nicotinamide
5531A	*pan-1*	requires pantothenic acid
F12 (74A-Y155-M39)	*ad-4*	requires adenine

* Information concerning the first five histidine strains, *tryp-1*, and *pan-1* is listed (with prior references) in BARRATT *et al.* (1954). Information on strains K-26 and K-34 is from MATHIESON and CATCHESIDE (1955). Information concerning the *ad-4* isolate can be found in GILES *et al.* (1957).

Heterocaryon complementation tests were carried out in the manner previously described by DE SERRES (1956). The over plating tests used in the genetic analyses were performed essentially in the manner described by NEWMEYER (1954).

Classification of mutants by heterocaryon tests

Initially 50 mutants from experiment Y155 (Table 2) were tested in all combinations by pairs in heterocaryon tests. Mutants which were mutually noncomplementary were placed in the same group, and a tester was arbitrarily selected to represent each group. Ultimately the 704 histidine mutants were divided into six major groups by further heterocaryon tests.

Since the previously described mutants (K-34 and representatives of groups *hist-1* through *hist-4*) were not compatible with the newly acquired histidine mutants, crosses were made with appropriate wild-type strains (74A or 3.1a) to introduce appropriate heterocaryon compatibility factors. With the heterocaryon-compatible strains thus obtained, tests were then made with representatives of each of the six heterocaryotically determined groups. Four of the heterocaryon groups were shown to be noncomplementary (presumably allelic) with the previously designated *hist-1*, *hist-2*, *hist-3*, and K-34 mutants. However, *hist-4* had no equivalent among the new groups by these tests and appears to be a distinct group.

The results of these heterocaryon complementation tests are summarized in Table 2 which indicates the classification of the mutants from the three different filtration experiments. Seven major groups of histidine-requiring mutants have thus been detected. Groups *hist-1* through *hist-4* have been previously designated by BARRATT *et al.* (1954). The additional new groups designated here are *hist-5*, and *hist-7*, with K-34 placed in the *hist-6* group.

TABLE 2

Classification of histidine mutants from three filtration concentration experiments

Experiment number	Wild type used	*hist-1*	*hist-2*	*hist-3*	*hist-4*	*hist-5*	*hist-6*	*hist-7*	Total
Y152	74A	7	8	8	0	15	13	2	53
Y155	74A	9	22	22	0	17	16	0	86
Y175	5.5A	39	41	82	0	80	76	10	328
Y175	3.1a	18	54	64	0	42	59	0	237
	Total	73	125	176	0	154	164	12	

Biochemical characteristics

Accumulation products of single histidine mutants: Physiological experiments were carried out in order to determine the biosynthetic step which is blocked in each of the new groups of mutants. The results of cross-feeding experiments in which mutants were incubated on minimal agar petri plates on opposite sides of a permeable cellophane membrane indicated that no compounds stimulatory to the growth of any histidine mutants were excreted by other histidine mutants. These results were in agreement with those of HAAS *et al.* (1952) in attempts to stimulate histidine mutants with culture filtrates and with synthetic supplements.

The evidence of AMES, MITCHELL and MITCHELL (1953) concerning the nature of the imidazole derivatives accumulated by representatives of various mutant groups and the biochemical evidence reviewed by AMES (1957) indicate the position of the biochemical blocks in certain of the groups. *hist-1* mutants are deficient for imidazoleglycerol phosphate dehydrase activity and accumulate imidazoleglycerol in growth medium. The *hist-2* mutant is blocked prior to imidazoleglycerol phosphate, as no imidazoles are accumulated in the medium during growth. *hist-3* mutants are heterogeneous, with C140 blocked early (accumulating no imidazoles) and T1710 accumulating L-histidinol and deficient for histidinol dehydrogenase activity. The *hist-4* mutant was shown by AMES (1957) to exhibit less than two percent of wild-type activity for histidinol phosphate phosphatase and to accumulate both imidazoleglycerol and imidazoleacetol in the medium during growth. The *hist-6* mutant, K-34 of MATHIESON and CATCHSIDE (1955), was found to accumulate no imidazoles and, hence, was placed prior to imidazoleglycerol phosphate in the histidine biosynthetic scheme.

PAULY's (1904) tests for imidazoles and BRATTON-MARSHALL (1939) tests for diazotizable amines were carried out to determine whether imidazoles or diazotizable amines were accumulated by representatives of the mutant groups and to determine the biochemical characteristics of each of the new histidine mutant groups. For this purpose, representatives of each group were grown in 2×15 cm test tubes on 5 ml each of Fries' liquid medium supplemented with 25 micrograms/ml of L-histidine HCl. With this medium the histidine supply is limiting, and the exogenous histidine supply is usually exhausted within seven days. The mycelial pads were squeezed dry and autoclaved briefly in 0.5–1.0 ml of water. The resulting liquid was spotted on Whatman No. 1 filter paper and

developed as an ascending chromatogram with either the acidic or basic solvent of HAAS *et al.* (1952). Spots of imidazole derivatives were detected by spraying with Pauly reagents as described by MATHIESON and CATCHSIDE (1955). The three most conspicuous imidazole derivatives, detected at approximately the Rf's indicated by AMES *et al.* (1953) and compared with synthetic histidinol and histidine or with accumulant spots from known mutants, were imidazoleglycerol, imidazoleacetol, and histidinol. In some samples light spots of unused histidine were also found. The phosphate derivatives of these compounds were not observed. Separate chromatograms were tested for diazotizable amines by modification of the Bratton-Marshall test in which the paper is sprayed first with 0.2 percent sodium nitrite in 0.1 N HCl, dried, and then sprayed with 0.2 percent N(1-naphthyl)-ethylene diamine HCl in 95 percent ethanol. The filtrates from the above cultures were also tested directly by Pauly tests as described by AMES and MITCHELL (1955) and by Bratton-Marshall tests as described by STEWART and SEVAG (1952).

The results of Pauly tests and Bratton-Marshall tests of filtrates from single histidine mutants are presented in Table 3. These results are consistent with those of previous workers although additional groups are studied here, and the

TABLE 3

Compounds accumulated in growth medium by representative mutants from the seven histidine groups

Mutant group	Representative mutants	Tests of whole filtrates		Chromatogram tests				Position of block in biosynthetic scheme
		Pauly	Bratton-Marshall	Bratton-Marshall	Imidazoleglycerol	Imidazoleacetol	Histidinol	
Wild type	74A	—	—	—	—	—	—	none
hist-1	Y155-M302	+	—	—	+	—	—	between imidazoleglycerol and imidazoleacetol
hist-2	Y152-M43	—	—	—	—	—	—	before imidazoleglycerol
hist-3 (nonacc)*	C140, Y152-M111, Y193-M16	—	—	—	—	—	—	before imidazoleglycerol
hist-3 (acc)†	T1710, Y155–M234, Y155-M261	+	—	—	—	—	+	after histidinol
hist-4	C141	+	—	—	+	+	—	between imidazoleacetol and histidinol
hist-5	Y152-M108	+	—	—	+	+	—	between imidazoleacetol and histidinol
hist-6	Y152-M105	—	+	+	—	—	—	before imidazoleglycerol
hist-7	Y152-M31	—	+	+	—	—	—	before imidazoleglycerol

* Nonaccumulator.
† Accumulator.

information from Bratton-Marshall tests is new. Certain *hist-3* mutants characteristically accumulate histidinol while others consistently accumulate no detectable imidazoles or diazotizable amines. The two groups of mutants which accumulate no Pauly or Bratton-Marshall positive compounds are the *hist-3* nonaccumulators and *hist-2* mutants. There are two groups, *hist-6* and *hist-7*, which accumulate only a diazotizable amine. *hist-1* mutants accumulate imidazoleglycerol in both the medium and mycelium. *hist-4* and *hist-5* mutants accumulate imidazoleglycerol and imidazoleacetol. *hist-4* has been shown by AMES (1957) to be deficient for histidinol phosphate phosphatase, hence the *hist-5* mutants may tentatively be assumed to lack imidazoleacetol phosphate transaminase activity. One class of *hist-3* mutants (deficient for histidinol dehydrogenase) accumulates histidinol.

Accumulation studies with double mutants: In tests for imidazole accumulation, HAAS *et al.* (1952) used double mutants, each containing two different histidine-requiring genes. A double mutant is expected to exhibit accumulation characteristics similar to those of the earlier of the two incorporated mutants in the biosynthetic scheme, and so the relative orders of pairs of genes in the histidine biosynthetic scheme may be tested by this means. Experiments were undertaken using this technique with Pauly and Bratton-Marshall tests in an attempt to establish the relative order of the early mutants which do not accumulate imidazoles (*hist-2*, *hist-3* nonaccumulators, *hist-6*, and *hist-7*) and to repeat the observations of HAAS *et al.* (1952) on *hist-3* accumulators and nonaccumulators with newly acquired *hist-3* mutants.

Progeny were obtained from appropriate crosses and double mutants selected by heterocaryon tests. The double mutants were grown on limiting histidine as described above, and the filtrates were tested by Pauly and Bratton-Marshall tests. The results of these tests for accumulants of double mutants are reported in Table 4.

TABLE 4

Tests of filtrates for compounds accumulated by double histidine mutants. (See Table 3 for controls, i.e., tests of filtrates of single mutants)

Genetic composition of double mutant strains	Imidazoles (Pauly tests)	Diazotizable amines (Bratton-Marshall tests)	Indicated order of two reactions blocked
hist-2 hist-7	—	—	*hist-2* before *hist-7*
hist-2 hist-1	—	—	*hist-2* before *hist-1*
hist-3 (Y152-M111) *hist-7*	—	—	*hist-3* before *hist-7*
hist-3 (Y152-M111) *hist-6*	—	—	*hist-3* before *hist-6*
hist-3 (Y152-M111) *hist-1*	—	—	*hist-3* before *hist-1*
hist-3 (Y152-M111) *hist-4*	—	—	*hist-3* before *hist-4*
hist-3 (Y193-M16) *hist-1*	—	—	*hist-3* before *hist-1*
hist-7 hist-1	—	+	*hist-7* before *hist-1*
hist-7 hist-5	—	+	*hist-7* before *hist-5*
hist-7 hist-4	—	+	*hist-7* before *hist-4*
hist-7 hist-3 (Y155-M261)	—	+	*hist-7* before *hist-3*
hist-6 hist-1	—	+	*hist-6* before *hist-1*
hist-6 hist-4	—	+	*hist-6* before *hist-4*

This evidence indicates that *hist-3* accumulators and nonaccumulators are blocked at different positions in the histidine biosynthetic scheme. The nonaccumulators (Y152-M111 and Y193-M16) are blocked before *hist-1* while evidence from doubles containing Y152-M111 indicates a position before *hist-6* and *hist-7* for this nonaccumulator. Y155-M261 appears from this evidence to be located no earlier than *hist-1* while the fact that it accumulates only histidinol (Table 3) places it in the terminal step.

The earliest genetic blocks, exhibited by *hist-3* nonaccumulators and *hist-2* mutants, result in a lack of accumulation of imidazoles and of diazotizable amines. The relative order of the two groups cannot be determined by present tests. Next in the biosynthetic scheme are the *hist-6* and *hist-7* mutants, which accumulate a diazotizable amine. Again it is impossible to determine the relative order of these two groups. The order of the remaining mutant groups is *hist-1*, *hist-5*, *hist-4* and *hist-3* accumulators, with the assignment of *hist-5* tentative, as discussed above. The order of these mutant groups in the biosynthetic scheme for histidine is summarized in Figure 1.

Genetic studies

The purpose of genetic studies with the histidine mutants is to confirm the identity of representatives from each of the seven major physiological groups described above and to test whether two groups of mutants, which appear on the basis of heterocaryon complementation tests to be dissimilar, actually might be allelic mutants exhibiting interallelic complementation (GILES, PARTRIDGE and NELSON 1957). Of special interest in this respect are the pairs of groups which exhibit indistinguishable accumulation characteristics. The determination of the genetic position of groups previously undetected may also facilitate the use of

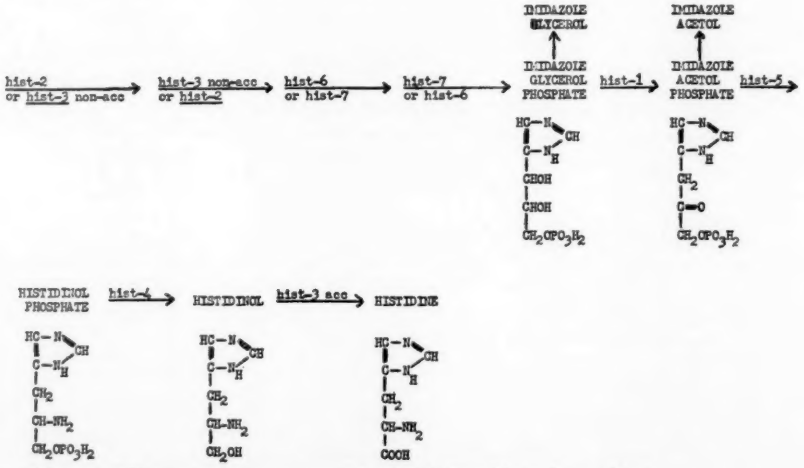

FIGURE 1.—Placement of mutant groups in the histidine biosynthetic scheme.

these mutants as markers for later crossing experiments. The genetic information concerning these mutants is listed in Table 5 and will be discussed for each group in the following paragraphs.

hist-1: This group was first assigned to linkage group V by HAAS *et al.* (1952). GILES (1955) reported evidence leading to the following order and map distances (in parenthesis) for certain linkage group V markers: centromere (10) isoleucinevaline (*iv-1*) (10) *hist-1* (7) inositol (*inos*) (3) para-aminobenzoic acid

TABLE 5

Genetic analysis for linkage relationship of histidine groups

Cross no.	Isolation numbers and genetic constitution of strains crossed	Estimate of viable spores plated on selective medium	Supplement in selective medium	Colonies in selective medium	Percent *hist*+ isolates*
1	Y152-M43 (*hist-2*)	2080	arginine	69	3.3
	74-YU938-1a (*arg-3 nic-2*)	2080	nicotinamide	41	2.0
		2080	none	0	
2	Y152-M14 (*hist-2*)	3770	arginine	139	3.7
	74-YU938-1a (*arg-3 nic-2*)	3770	nicotinamide	77	2.0
		3770	none	6	
3	Y193-M16 (*hist-3* nonacc)	7500	arginine	95	1.3
	74-YU938-1a (*arg-3 nic-2*)	7500	nicotinamide	213	2.8
		7500	none	9	
4	Y155-M261 (*hist-3* acc)	2960	arginine	68	2.3
	74-YU938-1a (*arg-3 nic-2*)	2960	nicotinamide	95	3.2
		2960	none	1	
5	C140 (*hist-3* nonacc)	5310	none	0	0
	K26a (*hist-3* acc)				
6	74-OR14-6A (*ad-5 nic-2*)	1540	nicotinamide	73	4.7
	K26a (*hist-3* acc)	1540	adenine	23	1.5
7	Y152-M43 (*hist-2*)	10518	none	172	1.7
	K26a (*hist-3* acc)				
8	5531A (*pan-1*)	7336	none	488	6.7
	C141a (*hist-4*)				
9	Y152-M108 (*hist-5*)	2700	none	693	25.7
	C141a (*hist-4*)				
10	Y152-M1 (*hist-6*)	610	none	0	0
	K34a (*hist-6*)				
11	Y152-M31 (*hist-7*)	1721	none	161	9.4
	Y155-M39 (*ad-4*)				
12	5.5A-Y175-M275 (*hist-7*)	1217	none	133	10.9
	Y155-M39 (*ad-4*)				
13	Y152-M31 (*hist-7*)	4341	none	92	2.1
	10575a (*tryp-1*)				
14	Y193-M10 (*hist-7*)	6410	none	111	1.7
	10575a (*tryp-1*)				
15	Y152-M1 (*hist-6*)	5610	none	1278	22.8
	10575a (*tryp-1*)				
16	Y152-M105 (*hist-6*)	3525	none	533	15.1
	10575a (*tryp-1*)				

* Double for conventional map units.

(*pab-1*). More recent crosses (CASE, unpublished) between an *iv-1 inos* double mutant and numerous *hist-1* mutants, which were classified by heterocaryon tests in the experiments discussed here, indicate that these *hist-1* mutants are also clearly located between the *iv-1* and *inos* markers.

hist-2: The *hist-2* locus was shown by HAAS *et al.* (1952) to be in the right arm of linkage group I. The present evidence from crosses no. 1 and 2 between newly acquired *hist-2* mutants and a common *arg-3 nic-2* double indicates that *hist-2* is located between the other two markers and about seven map units to the left of *nic-2*.

hist-3: The *hist-3* locus was shown by HAAS *et al.* (1952) to be in the right arm of linkage group I. Crosses no. 3 and 4 (an *arg-3 nic-2* strain to an accumulating and a nonaccumulating *hist-3* mutant) provide evidence that both *hist-3* mutants are between the other two markers and about three map units to the left of *nic-2*. Cross no. 5 between C140 (*hist-3* by definition) of HAAS *et al.* (1952) and K-26 of MATHIESON and CATCHESIDE (1955) supports the heterocaryon evidence that K-26 is a *hist-3* mutant. Cross no. 6 further confirms this by showing that K-26 is similar to other *hist-3* mutants in crosses to an *ad-5 nic-2* double.

Since *hist-2* mutants and *hist-3* nonaccumulators have indistinguishable accumulation characteristics, it is important to consider whether these two groups are allelic. The first four crosses in Table 5 indicate that the distance between *hist-2* and *nic-2* is about six map units while the *hist-3* to *nic-2* distance is about three units. Cross no. 7 between a *hist-2* and a *hist-3* mutant indicates a map distance between the two loci of 3.2 units. The unpublished evidence of DE SERRES that the *lys-4* locus is situated between *hist-2* and *hist-3* seems to rule out effectively the idea of a single long continuous locus. The evidence indicates that *hist-2* and *hist-3* are linked but nonallelic.

hist-4: The *hist-4* locus has been placed by HAAS *et al.* (1952) in linkage group IV. MITCHELL and MITCHELL (1954) have found it to be located about six units distal to *cot* (colonial-temperature sensitive) in the right arm of linkage group IV. No new mutants were obtained in this group; however, cross no. 8, involving strain C141, shows a *pan-1* to *hist-4* distance of 13.4 map units, which is not inconsistent with the former placement.

hist-5: The *hist-5* group, not previously detected in Neurospora, has similar accumulation characteristics to *hist-4*. This group has not been located genetically. Evidence that the group is different genetically from *hist-4* is found in cross no. 9 which indicates that *hist-4* and *hist-5* exhibit random assortment.

hist-6: A *hist-6* mutant (K-34) was located by MATHIESON and CATCHESIDE (1955) 32.6 units from *pan-1* in linkage group IV. This placement was not further investigated here. Evidence from cross no. 10 confirms the heterocaryon evidence that K-34 is a *hist-6* mutant.

hist-7: The *hist-7* group has been located by crosses no. 11 through 14 in linkage group III about 20 units from *ad-4* and four units from *tryp-1*.

hist-6 and *hist-7* representatives exhibit similar accumulation characteristics;

however, evidence that these are nonallelic is presented in crosses no. 15 and 16, which show that two *hist-6* mutants are clearly not closely linked to *tryp-1* and, therefore, not closely linked to *hist-7*.

The genetic experiments thus confirm the classification derived from heterocaryon complementation tests and provide evidence that, although some of the mutant groups are not distinguishable by tests of accumulants, they are indeed nonallelic.

DISCUSSION

Characterization of mutant groups: The classification of 704 histidine-requiring mutants into seven major groups on the basis of heterocaryon complementation tests has been described. Of the seven groups, four *(hist-1* through *hist-4)* were previously described by HAAS *et al.* (1952) and given group designations by BARRATT *et al.* (1954). An additional group was described by MATHIESON and CATCHESIDE (1955) and is now designated *hist-6*. Of the two new mutant groups, one *(hist-5)* has been tentatively assumed to lack imidazoleacetol phosphate transaminase activity; the other *(hist-7)* has been placed prior to imidazoleglycerol phosphate in the biosynthetic scheme and located in linkage group III. The enzymatic steps between imidazoleglycerol phosphate and histidine reviewed by AMES (1957), i.e., imidazoleglycerol phosphate dehydrase, imidazoleacetol phosphate transaminase, histidinol phosphate phosphatase, and histidinol dehydrogenase, are considered lacking in the mutant groups *hist-1*, *hist-5*, *hist-4*, and *hist-3* accumulators, respectively. Four mutant groups (i.e., *hist-2*, *hist-3* nonaccumulators, *hist-6* and *hist-7*) are placed prior to imidazoleglycerol phosphate in the biosynthetic scheme.

Bratton-Marshall tests were used to detect a diazotizable amine accumulated by representatives of the *hist-6* and *hist-7* groups in growth medium. Evidence has been obtained to indicate that *hist-6* and *hist-7* mutants are blocked later in the biosynthetic scheme than are the *hist-3* nonaccumulators and the *hist-2* mutants.

The genetic experiments have provided evidence that representatives of certain heterocaryon groups which are indistinguishable by accumulation tests are indeed nonallelic. This supports the contention that seven distinct groups are available by ruling out the possibility that any of these pairs of mutant groups should be classified as a single group which exhibits an interallelic complementation pattern.

Absence of hist-4 *mutants:* The striking fact that no *hist-4* mutants have been obtained in these filtration concentration experiments raises the quesion whether *hist-4* might be only one of a number of additional groups of histidine mutants not obtained in these experiments and hence not yet studied. One possible explanation for the absence of *hist-4* mutants is that while C141 (the only *hist-4* mutant) was derived from ultraviolet irradiated conidia, no ultraviolet histidine mutants were screened systematically in the present experiments. On the other hand, AMES (1957) has pointed out that C141, although deficient for histidinol

phosphate phosphatase activity, grows slightly on minimal medium. He suggests that nonspecific phosphatases (inhibited by beryllium ions in the specific assay for histidinol phosphate phosphatase) in the mutant may convert histidinol phosphate to histidinol at a sufficient rate to account for the "leakiness" of C141. If this is a valid suggestion, then all mutants specifically lacking histidinol phosphate phosphatase activity might be expected to be more or less "leaky" and perhaps to grow sufficiently to cause their loss in filtration concentration experiments. *hist-4* mutants may thus, by their very nature, be unobtainable by the filtration concentration method without reflecting unfavorably upon the effectiveness of the method for obtaining a large majority of the mutant classes.

The fact that a substantial number of mutants were screened and no additional groups found suggests that probably all of the histidine mutant groups obtainable by filtration concentration experiments with X-irradiated conidia have been described.

Biochemistry of mutants before imidazoleglycerol phosphate: MOYED and MAGASANIK (1957, 1960) have indicated that the biosynthetic scheme for histidine begins with a reaction between ribose-5-phosphate and adenosine triphosphate to form an intermediate designated by them as compound III. This compound is then split, with the addition of an amino group from glutamine, to yield 5-amine-1-ribosyl-4-imidazolecarboxamide 5′-phosphate and imidazoleglycerol phosphate, the latter of which is converted through four enzymatic steps to histidine. The fact that only two reactions before imidazoleglycerol phosphate have previously been distinguished (with *Salmonella tryphimurium* mutants) and that four nonallelic Neurospora mutant groups are now shown to exhibit blocks before imidazoleglycerol phosphate is striking and may warrant further investigation.

hist-3 *mutants:* The present experiments, utilizing mutants derived from filtration concentration experiments, confirm the evidence of HAAS *et al.* (1952) that the *hist-3* genetic group is heterogenous with respect to physiological characteristics. The *hist-3* mutants may be tentatively separated into accumulators of histidinol and nonaccumulators (accumulating no imidazoles or diazotizable amines). The information from double mutants indicates that nonaccumulators are blocked early in the biosynthetic scheme while histidinol accumulators are blocked later. These findings have led to experiments involving intensive complementation tests and interallelic crosses of *hist-3* mutants. This work will be described in subsequent papers.

SUMMARY

1. Seven hundred and four histidine-requiring Neurospora mutants obtained from filtration concentration experiments have been classified by heterocaryon complementation tests into seven physiological groups. Two groups previously undescribed and one briefly described were assigned the designations *hist-5*, *hist-6*, and *hist-7*. No representatives of the previously described *hist-4* group were found among the mutants studied.

2. The previous subdivision of the *hist-3* groups by HAAS *et al.* (1952) into distinct biochemical classes of mutants has been supported by accumulation

studies with new mutants. Similar evidence has permitted the placement of four classes of mutants before imidazoleglycerol phosphate in the biosynthetic scheme and four after imidazoleglycerol phosphate.

3. Genetic analysis has resulted in the placement of the *hist-7* group in linkage group III. Representatives of certain mutant groups which accumulate similar intermediates have been characterized by genetic tests as nonallelic.

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LITERATURE CITED

AMES, B. N., 1957 The biosynthesis of histidine; L-histidinol phosphate phosphatase. J. Biol. Chem. **226**: 583–593.

AMES, B. N., and H. K. MITCHELL, 1955 The biosynthesis of histidine; imidazoleglycerol phosphate, imidazoleacetol phosphate, and histidinol phosphate. J. Biol. Chem. **212**: 687–696.

AMES, B. N., H. K. MITCHELL, and M. B. MITCHELL, 1953 Some new naturally occurring imidazoles related to the biosynthesis of histidine. J. Am. Chem. Soc. **75**: 1015–1018.

BARRATT, R. W., D. NEWMEYER, D. D. PERKINS, and L. GARNJOBST, 1954 Map construction in *Neurospora crassa*. Advances in Genet. **6**: 1–93.

BRATTON, A. C., and E. K. MARSHALL, 1939 A new coupling component for sulfanilamide determination. J. Biol. Chem. **128**: 537–550.

CASE, M. E., and N. H. GILES, 1958 Recombination mechanisms at the *pan-2* locus in *Neurospora crassa*. Cold Spring Harbor Symposia Quant. Biol. **23**: 119–134.

DE SERRES, F. J., 1956 Studies with purple adenine mutants in *Neurospora crassa*. I. Structural and functional complexity in the *ad-3* region. Genetics **41**: 668–676.

GILES, N. H., 1955 Forward and back mutation at specific loci in Neurospora. Brookhaven Symposia In Biol. **8**: 103–125.

GILES, N. H., C. W. H. PARTRIDGE, and N. H. NELSON, 1957 The genetic control of adenylosuccinase in *Neurospora crassa*. Proc. Natl. Acad. Sci. U.S. **43**: 305–317.

HAAS, F., M. B. MITCHELL, B. N. AMES, and H. K. MITCHELL, 1952 A series of histidineless mutants of *Neurospora crassa*. Genetics **37**: 217–226.

MATHIESON, M. J., and D. G. CATCHESIDE, 1955 Inhibition of histidine uptake in *Neurospora crassa*. J. Gen. Microbiol. **13**: 72–83.

MITCHELL, M. B., and H. K. MITCHELL, 1954 A partial map of linkage group D in *Neurospora crassa*. Proc. Natl. Acad. Sci. U.S. **40**: 436–440.

MOYED, H. S., and B. MAGASANIK, 1957 The role of purines in histidine biosynthesis. J. Am. Chem. Soc. **79**: 4812–4813.

1960 The biosynthesis of the imidazole ring of histidine. J. Biol. Chem. **235**: 149–153.

NEWMEYER, D., 1954 A plating method for genetic analysis in Neurospora. Genetics **39**: 604–618.

PAULY, H., 1904 Uber die Konstitution des Histidins. Z. Physiol. Chem. **42**: 508–518.

STEWART, R. C., and M. G. SEVAG, 1952 4-Amino-5-imidazolecarboxamide. Role of carbohydrates as critical factor for its accumulation. Arch. Biochem. Biophys. **41**: 9–13.

GENETICAL AND BIOCHEMICAL STUDIES OF HISTIDINE-REQUIRING MUTANTS OF NEUROSPORA CRASSA. II. EVIDENCE CONCERNING HETEROGENEITY AMONG hist-3 MUTANTS[1]

BROOKE B. WEBBER[2]

Department of Botany, Josiah Willard Gibbs Research Laboratories, Yale University, New Haven, Connecticut

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THE *hist-3* mutants of *Neurospora crassa* were chosen for extensive genetic analysis because of the finding by HAAS, MITCHELL, AMES and MITCHELL (1952) that two *hist-3* mutants, T1710 and C140, though genetically allelic, appeared to be physiologically dissimilar. T1710 was shown to accumulate during growth a single imidazole, L-histidinol (AMES, MITCHELL and MITCHELL 1953), while C140 accumulates no compound detectable by the PAULY (1904) test for imidazole derivatives. Furthermore, C140, when combined as a double mutant with other nonallelic histidine mutants, appeared to be blocked very early in the biosynthetic sequence for histidine (i.e., before imidazole glycerol phosphate). T1710, on the other hand, behaved in such doubles as though blocked in the terminal step in histidine biosynthesis. These findings were confirmed by WEBBER and CASE (1960) with a series of newly acquired *hist-3* mutants. Genetic experiments (WEBBER, unpublished) involving interallelic crosses of a variety of *hist-3* mutants have placed all within a genetic region 0.03 map units in length.

In the present studies the *hist-3* nonaccumulators have been further subdivided into two groups. These two nonaccumulator groups and the accumulators constitute the three major subgroups of *hist-3* mutants which have been characterized as follows: (subgroup 1) histidinol accumulators; (subgroup 2) nonaccumulators of imidazoles which complement in heterocaryons with all subgroup 1 mutants; (subgroup 3) nonaccumulators which complement with no subgroup 1 mutants. The hypothesis was advanced that these represent respectively: (1) mutants deficient for histidinol dehydrogenase activity; (2) mutants deficient for some unidentified enzymatic activity early in histidine biosynthesis; and (3) mutants deficient for both activities. An additional class of mutants, characterized by slight histidinol accumulation, was thought to be intermediate between subgroup 1 and 3, lacking histidinol dehydrogenase activity and having only weak

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[2] Present address: Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee.

activity for the early reaction. The present paper describes experiments which support this characterization of the *hist-3* subgroups.

Initially crosses were made to determine whether these differences in behavior with respect to imidazole accumulation are allele specific or whether alterations in accumulation characteristics can be produced in given *hist-3* alleles by appropriate crosses. Early studies by HAAS *et al.* (1952) and by WEBBER and CASE (1960) involving interallelic heterocaryon tests with these mutants indicated that subgroup 1 and subgroup 3 mutants, although different in accumulation characteristics, are noncomplementary in heterocaryon tests. Experiments have now been designed to test whether lack of complementation might be due to failure of initial heterocaryon formation or to a peculiar incompatibility reaction. Further observations of accumulation characteristics were made to characterize mutants from the three subgroups.

The early reactions in histidine biosynthesis have only recently been studied *in vitro* (MOYED and MAGASANIK 1960); however, a histidinol dehydrogenase assay method has been developed by ADAMS (1954) and modified by AMES, GARRY and HERZENBERG (1960). This was employed to determine whether mutants representing each of the *hist-3* subgroups exhibit histidinol dehydrogenase activity. A discussion of these experiments designed to characterize the three subgroups of *hist-3* mutants follows.

MATERIALS AND METHODS

The origin of the heterocaryon compatible histidine mutants from filtration concentration experiments has been described by WEBBER and CASE (1960). Heterocaryon complementation tests were carried out as previously described by DE SERRES (1956). For tests of imidazole accumulation, test tubes (2 × 15 cm), each containing 5 ml of Fries' liquid medium supplemented with 25 micrograms/ml of L-histidine-HCl, were inoculated with appropriate mutant conidia or heterocaryotic mycelial transfers, and the tubes were incubated for 7–10 days at 25°C. Aliquots of the medium were then tested by Pauly tests for imidazoles as described by AMES and MITCHELL (1955). Imidazole accumulators excrete into the medium sufficient imidazoles to produce a red color in Pauly tests stronger than that produced by 25 micrograms/ml of L-histidine-HCl (as determined by eye and representative testings in a colorimeter) and about equivalent to the color produced by 100–200 micrograms/ml of L-histidine-HCl. Nonaccumulators appear to utilize the supplementary histidine and so leave the medium with less than the 25 micrograms/ml initially added.

Histidinol dehydrogenase assays were carried out as described by AMES, GARRY and HERZENBERG (1960). Diaphorase catalyzes the reduction of dichlorophenol indophenol dye by the DPNH formed in the oxidation of histidinol. The dye reduction was read in a Cary spectrophotometer and enzyme activity expressed as rate (decrease in optical density at 600 millimicra per hour) per mg protein. Neurospora extract for the assay was prepared by shaking for 60 seconds at 0°C in a Nossal shaker 2 gm acid-washed glass beads, 2 ml of 0.05 M triethanolamine

buffer at *p*H 7.5, and 1 gm of frozen Neurospora mycelium (grown for three days at 25°C in stationary culture on Fries' liquid medium supplemented with 25 micrograms/ml L-histidine-HCl). The broken cell extract was then centrifuged for 15 minutes at 25,000 g. The supernatant was dialyzed at 4°C for 2.5 hrs against two changes of 1500 ml of *p*H 7.5 0.01 M triethanolamine-HCl buffer. The extract usually contained 4–10 mg protein/ml as indicated by the phenol method of SUTHERLAND, CORI, HAYNES and OLSEN (1949). 0.01–0.05 ml of extract were used per assay.

RESULTS

Subgroups of hist-3 *mutants:* Early experiments indicated that all *hist-3* nonaccumulators of imidazoles failed to complement with any other *hist-3* mutant. However, in further routine heterocaryon tests for interallelic complementation among *hist-3* mutants, it was found that two mutants (Y193-M16 and Y193-M17), both nonaccumulators of imidazoles, complemented with a number of other *hist-3* mutants to form a histidine-independent heterocaryon. Further tests showed that all accumulators complemented with Y193-M16 and Y193-M17 while all nonaccumulators, then available, failed to complement with these two mutants. This correlation between accumulation and ability to complement with Y193-M16 and Y193-M17 was consistent to the extent that certain *hist-3* mutants which exhibit slight imidazole accumulation (e.g., Y152-M16 and Y226-M579) exhibit also a delayed and slow-growing response in complementation tests with Y193-M16. Accordingly, three major subgroups of *hist-3* mutants were designated as follows: (subgroup 1) histidinol accumulators; (subgroup 2) nonaccumulators (e.g., Y193-M16 and Y193-M17) which complement with subgroup 1 mutants; and (subgroup 3) nonaccumulators which fail to complement with subgroup 1 mutants. This was tentatively explained by the hypothesis that the two complementary subgroups (1 and 2) comprise mutants deficient for one or the other of two separate enzyme activities while subgroup 3 comprises mutants deficient for both reactions. The *hist-3* mutants characterized by slight histidinol accumulation are considered deficient for the later of the two reactions and "leaky" for the earlier reaction; these will be referred to subsequently as weak accumulators or "weak subgroup 1" mutants.

In Table 1 are listed the *hist-3* mutants used in subsequent experiments arranged by presumptive subgroup characterizations arrived at by accumulation and heterocaryon tests. Included are some recently acquired subgroup 2 mutants, used in tests for allele specificity of accumulation characteristics, which exhibit intrasubgroup complementation with Y193-M16. Since the subgroup characterizations of nonaccumulators is based only upon heterocaryon evidence, they must be considered tentative and subject to correction by the findings of suitable *in vitro* assays.

AMES (1957) has shown two *hist-3* accumulators (i.e., T1710 of HAAS *et al.* 1952, and K-26 of MATHIESON and CATCHESIDE 1955) to be deficient for histidinol dehydrogenase activity. HAAS *et al.* (1952), using the subgroup 3 mutant C140, have shown the other enzyme activity to be prior to imidazoleglycerol phosphate

TABLE 1

hist-3 *mutants used*

Isolate number	Imidazole accumulation (Pauly tests)	Heterocaryon tests with Y193-M16 (subgroup 2)	Heterocaryon tests with Y155-M275 (subgroup 1)	Used in forced heterocaryon tests* Marked with *pan-2*	Used in forced heterocaryon tests* Marked with *nic-2*
(Presumptive subgroup 1 mutants)					
T1710	+	+	—	.	x
Y152-M51	+	+	—	.	x
Y155-M224	+	+	—	.	x
Y155-M226	+	+	—	x	.
Y155-M234	+	+	—	x	.
Y155-M245	+	+	—	x	x
Y155-M261	+	+	—	.	x
Y155-M268	+	+	—	x	.
Y155-M272	+	+	—	.	x
Y155-M275	+	+	. .	x	x
Y155-M280	+	+	—	.	x
Y226-M566	+	+	—	.	.
Y189-M93	+	+	—	.	x
(Presumptive subgroup 2 mutants)					
Y193-M16	—	. .	+	x	.
Y193-M17	—	—	+	x	.
Y224-M24	—	+	+	.	.
Y226-M584	—	+	+	.	.
Y226-M589	—	+	+	.	.
Y226-M606	—	+	+	.	.
(Presumptive subgroup 3 mutants)					
C140	—	—	—	.	x
Y152-M9	—	—	—	.	x
Y152-M35	—	—	—	.	x
Y152-M53	—	—	—	.	x
Y152-M66	—	—	—	x	x
Y152-M111	—	—	—	x	x
Y155-M225	—	—	—	x	x
Y155-M232	—	—	—	.	x
Y155-M233	—	—	—	x	.
Y155-M267	—	—	—	x	.
Y155-M270	—	—	—	.	x
Y155-M278	—	—	—	x	.
Y155-M283	—	—	—	x	x
Y155-M304	—	—	—	x	x
Y155-M305	—	—	—	x	.
Y226-M565	—	—	—	.	.
Y226-M585	—	—	—	.	.
Y189-M88	—	—	—	.	x
Y189-M95	—	—	—	.	x
(Slight accumulators—"weak subgroup 1")					
Y152-M16	slight	slow	—	.	x
Y226–M579	slight	slow	—	.	.

* Mutants marked x were used in forced heterocaryon tests described in the text under the section heading *Forced heterocaryon tests with noncomplementary* hist-3 *mutants*. Mutants not marked with x were not used in such tests.

in the histidine biosynthetic scheme. WEBBER and CASE (1960) have shown with a subgroup 3 mutant (Y152-M111) that the early enzyme activity probably represents the first or second of four steps prior to imidazoleglycerol phosphate and have demonstrated that a subgroup 2 mutant (Y193-M16) behaves in a double histidine strain as expected if it is blocked before imidazoleglycerol phosphate. This indicates that the two enzyme activities for which the subgroup 3 mutants are deficient are histidinol dehydrogenase and some reaction early (before imidazoleglycerol phosphate) in the histidine biosynthetic scheme.

Allele specificity of accumulation characteristics: To test whether the accumulation characteristics of *hist-3* mutants are characteristics of the particular *hist-3* alleles or whether they result from modifiers at other loci, a number of *hist-3* mutants were each crossed to a common strain (74-YU938-1a) bearing the linked markers *arg-3* (arginine requiring) and *nic-2* (niacin requiring) which are 6–7 units to the left and 2–3 units to the right, respectively, of *hist-3*. From these crosses, *hist-3 arg-3* double mutant progeny (presumed to have arisen by crossovers within a short distance to the left of *hist-3*) and *hist-3 nic-2* double mutant progeny (resulting presumably from crossovers within a short distance to the right of *hist-3*) were isolated by selective plating of ascospores and identified by testing for growth requirements. By these isolation procedures genetic material from the *arg-3 nic-2* strain was introduced into the *hist-3* strain by crossing over and independent assortment. The *hist-3 arg-3* and *hist-3 nic-2* double mutant isolates were then tested for imidazole accumulation as described above.

Thirty-seven *hist-3 arg-3* progeny and 32 *hist-3 nic-2* progeny, thus selected from crosses involving various *hist-3* parents, exhibited the same accumulation characteristics as their parents. No strains with exceptional accumulation characteristics were found among the progeny tested. Table 2 lists the number of *hist-3 nic-2* double mutant progeny, the number of *hist-3 arg-3* double mutant progeny selected and tested from each cross, and indicates the accumulation characteristic

TABLE 2

hist-3 *mutants and number of progeny used in tests of allele specificity of accumulation characteristics (see text)*

Hist-3 mutant crossed (to *arg-3 nic-2* double mutant strain)	Imidazole accumulation (Pauly test results) by parent and all *hist-3* progeny	Number of *hist-3 arg-3* doubles tested	Number of *hist-3 nic-2* doubles tested
Y226-M585	—	2	2
Y226-M565	—	10	0
Y226-M584	—	6	5
Y226-M606	—	3	4
Y226-M589	—	0	5
Y224-M24	—	4	3
Y226-M579	slight	5	5
Y226-M566	+	3	5
Y155-M234	+	4	3
	Total	37	32

of the parental *hist-3* mutant and all progeny tested from each cross. Since selective procedures designed to insure alterations in the genetic background of *hist-3* mutants did not cause modification of the accumulation characteristics of the *hist-3* mutant, it is assumed that the genetic material controlling the accumulation is at or very closely linked to the *hist-3* locus.

Forced heterocaryon tests with noncomplementary hist-3 *mutants:* The failure of subgroup 1 mutants (histidinol accumulators) to complement in heterocaryon tests with subgroup 3 mutants (nonaccumulators) presented an interesting problem. In order to test whether this lack of complementation was a result of failure of initial hyphal fusion or of a peculiar incompatibility reaction unrelated to the *hist-3* situation, experiments were undertaken to force such heterocaryons on histidine-supplemented medium by means of independent biochemical markers and then test for growth in the absence of histidine.

Five subgroup 1 strains, two mutants from subgroup 2, and nine from subgroup 3 were marked by appropriate crosses with *pan-2* (pantothenic acid requiring); nine mutants from subgroup 1 and 13 from subgroup 3 were marked with *nic-2* (niacin requiring) by crosses with 74-YU938-1a *(arg-3 nic-2)*. The *hist-3* mutants used are indicated in Table 1. Conidial suspensions of these double mutant strains were inoculated onto Petri dishes of Fries' agar medium supplemented with 25 micrograms/ml of L-histidine-HCl in all possible pairwise combinations such that one strain of each pair was marked with *nic-2* and the other with *pan-2*. Growth was vigorous, and after one day small blocks of agar containing heterocaryotic mycelium were transferred from the growing edge of each Petri dish to new Petri dishes of Fries' minimal agar medium. These latter plates were incubated at 25°C and observed daily. Growth was observed in control plates containing mutants of subgroup 1 with subgroup 2, as well as in certain pairs of subgroup 1 strains which consistently exhibit an intrasubgroup complementation response (WEBBER, unpublished). In all other cases histidine supplementation from the inoculum agar block was sufficient to permit slight growth on the minimal plates, but this growth soon ceased. The occurrence of vigorous growth in the histidine supplemented plates indicates that incompatibility factors are not involved while the cessation of growth on minimal agar confirms the prior observation that subgroup 1 and subgroup 3 mutants, although exhibiting different accumulation characteristics, are incapable of producing a histidine-independent heterocaryon.

Accumulation characteristics of forced heterocaryons: Tests were designed to determine whether a forced heterocaryon containing a subgroup 1 mutant (accumulator) and a subgroup 3 mutant (nonaccumulator) would accumulate an imidazole derivative. Forced heterocaryons were established with *pan-2* and *nic-2* as independent biochemical markers as previously described. Inocula were then grown on suitable liquid medium (see MATERIALS AND METHODS) and tested for imidazole accumulation.

All subgroup 1–subgroup 3 pairs tested were shown to be strong accumulators of Pauly detectable material. This finding is consistent with the hypothesis that subgroup 1 and subgroup 3 mutants are both deficient for histidinol dehydro-

genase and that subgroup 3 mutants additionally are deficient for an enzyme activity early in histidine biosynthesis which is provided by the subgroup 1 nuclei in the heterocaryons tested here. The finding does not support the idea that the early block is a secondary effect resulting from inhibition by a compound accumulated by a primary block late in histidine biosynthesis; if inhibitors were involved, they would be expected to be effective in the heterocaryons studied here.

Histidinol dehydrogenase assays: In vitro assays for histidinol activity were carried out as described in MATERIALS AND METHODS to provide more direct evidence concerning the characteristics of the three *hist-3* subgroups. One would expect to be able to characterize any *hist-3* mutant on the basis of *in vitro* assays and accumulation characteristics as follows: (subgroup 1) histidinol accumulators, deficient for histidinol dehydrogenase; (subgroup 2) nonaccumulators which exhibit histidinol dehydrogenase activity; (subgroup 3) nonaccumulators deficient for histidinol dehydrogenase activity.

The results of the assays are presented in Table 3. The sensitivity of the assay used with unfractionated mycelial homogenates is such that "no measurable activity" in Table 3 may be interpreted as less than about 20 percent of wild-type activity. The evidence indicates that subgroup 1 and subgroup 3 homogenates exhibit less than 20 percent of wild-type activity while subgroup 2 mutants exhibit activity quantitatively equivalent to or greater than wild type.

TABLE 3

Histidinol dehydrogenase assay data

Mutant classification	Mutant isolate number	Enzyme activity
Wild type	74A	6.6
	74A	7.0
	74A	7.6
	74A	9.8
	74A	8.5
	74A	7.6
hist-5	Y152-M108	8.6
hist-3 subgroup 1	Y155-M261	none
	Y155-M234	none
	Y155-M245	none
	Y155-M275	none
hist-3 subgroup 2	Y193-M16	11.6
	Y193-M16	17.6
	Y193-M17	8.6
	Y193-M17	9.0
hist-3 subgroup 3	Y152-M111	none
	Y155-M283	none
	Y152-M66	none
hist-3 "weak subgroup 1"	Y152-M16	none

DISCUSSION

The present paper has presented evidence in support of the hypothesis that the *hist-3* group of mutants, though allelic in the classical sense, may be divided into the following three subgroups: (1) mutants deficient for histidinol dehydrogenase activity; (2) mutants deficient for some enzymatically controlled reaction early in histidine biosynthesis; (3) mutants deficient for both enzyme activities. This hypothesis has been supported by the following types of evidence. (1) Allele specific differences exist among *hist-3* mutants with respect to accumulation of imidazole intermediates. Subgroup 1 mutants accumulate histidinol while subgroup 2 and 3 mutants accumulate no imidazoles or diazotizable amines in their medium or mycelium during growth. (2) Evidence from tests for imidazoles accumulated by strains each containing two genes for histidine requirement indicate that nonaccumulating *hist-3* strains are blocked early in histidine biosynthesis while accumulators are blocked in the terminal step. (3) Subgroup 2 and 3 mutants, though similar with respect to accumulation characteristics, exhibit different complementation characteristics. Subgroup 2 mutants form histidine-independent heterocaryons with all subgroup 1 mutants while subgroup 3 mutants fail to complement with subgroup 1 mutants. (4) Forced heterocaryons between subgroup 1 and 3 mutants accumulate, as do subgroup 1 mutants alone, an imidazole derivative in growth medium. (5) Histidinol dehydrogenase activity was detected *in vitro* in homogenates of subgroup 2 mutants but not in homogenates of subgroup 1 or 3 mutants. Additional work (not described in the present paper; WEBBER, CASE and GILES, unpublished) with histidinol stimulation tests of *hist-3* strains rendered permeable to histidinol by genetic means indicates that subgroup 2 mutants possess the ability to convert histidinol to histidine (i.e., to grow on histidinol if permeable) while subgroup 3 and 1 mutants lack this ability.

A description of the genetic behavior of these mutants would be of interest. The subgroup 3 mutants (noncomplementary with both subgroup 1 and subgroup 2 mutants) appear to be deficient for two separate nonsequential reactions in histidine biosynthesis. The origin of such mutants as a result of two independent mutational events appears unlikely in view of the frequency of occurrence of subgroup 3 mutants. So either two separate loci may be inactivated by the same mutational event with considerable frequency or one locus controls these two distinct enzymatic activities in such a way as to permit their inactivation separately or jointly.

The existence of three distinct subgroups could be based on the existence of two distinct, but adjacent, loci concerned with enzymes for two distinct nonsequential reactions in histidine biosynthesis. Mutants at one locus (subgroup 1) would lack one activity; those at the second locus would lack the other activity; subgroup 3 mutants, which lack both activities, would then be presumed to carry genetic damage which causes the simultaneous loss of activity of adjacent parts of the two loci. CLOWES (1958a,b) has interpreted a situation involving cysteine requiring mutants of *Salmonella typhimurium* in this fashion, with doubly deficient mutants always behaving in genetic mapping experiments as deletion

types. Preliminary work has indicated that "reversion" to prototrophy does occur among certain *hist-3* subgroup 3 mutants; however, suppressor mutation has not yet been ruled out in this case.

An alternative interpretation for the Neurospora *hist-3* situation would be that these mutants arise by single mutational events which occur in three separate regions within a continuous locus. A study by ENGLESBERG and KILLEEN (1959) has revealed a situation in *E. coli* which appears to be of this type. The Neurospora *hist-3* locus could thus be viewed as consisting of three distinct regions. In addition to two genetic regions in which subgroup 1 and 2 mutants are located, there would be a third genetic region which can be damaged to produce subgroup 3 mutants. This third region would bear specificity either for part of a single bifunctional enzyme or for critical parts of two separate, but related, enzymes (in either case catalyzing two separate nonsequential reactions in histidine biosynthesis).

Experiments utilizing heterocaryon complementation tests and genetic mapping techniques in an effort to choose between these alternative interpretations are in progress and will be reported in a subsequent paper.

SUMMARY

1. The *hist-3* mutants of Neurospora, though allelic in the classical sense, may be divided into three subgroups characterized as follows: (1) mutants deficient for histidinol dehydrogenase activity; (2) mutants deficient for some unidentified activity early in histidine biosynthesis; (3) mutants deficient for both activities. Evidence is presented in support of these characterizations.

2. Possible implications of these findings with regard to genetic control at the *hist-3* region have been discussed briefly.

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LITERATURE CITED

ADAMS, E., 1954 The enzymatic synthesis of histidine from histidinol. J. Biol. Chem. **209**: 829–846.

AMES, B. N., 1957 The biosynthesis of histidine; L-histidinol phosphate phosphatase. J. Biol. Chem. **226**: 583–593.

AMES, B. N., B. GARRY, and L. A. HERZENBERG, 1960 The genetic control of the enzymes of histidine biosynthesis in *Salmonella typhimurium*. J. Gen. Microbiol. **22**: 369–378.

AMES, B. N., and H. K. MITCHELL, 1955 The biosynthesis of histidine: imidazoleglycerol phosphate, imidazoleacetol phosphate, and histidinol phosphate. J. Biol. Chem. **212**: 687–696.

AMES, B. N., H. K. MITCHELL, and M. B. MITCHELL, 1953 Some new naturally occurring imidazoles related to the biosynthesis of histidine. J. Am. Chem. Soc. **75**: 1015–1018.

CLOWES, R. C., 1958a Nutritional studies of cysteineless mutants of *Salmonella typhimurium*. J. Gen. Microbiol. **18**: 154–172.

1958b Investigation of the genetics of cysteineless mutants of *Salmonella typhimurium*. J. Gen. Microbiol. **18**: 154–172.

DE SERRES, F. J., 1956 Studies with purple adenine mutants in *Neurospora crassa*. I. Structural and functional complexity in the *ad-3* region. Genetics **41**: 668–676.

ENGLESBERG, E., and N. L. KILLEEN, 1959 Mixed gene loci controlling L-arabinose isomerase and L-ribulokinase activities in *Escherichia coli* B/r. (Abstr.) Genetics **44**: 508–509.

HAAS, F., M. B. MITCHELL, B. N. AMES, and H. K. MITCHELL, 1952 A series of histidineless mutants of *Neurospora crassa*. Genetics **37**: 217–226.

MATHIESON, M. J., and D. G. CATCHESIDE, 1955 Inhibition of histidine uptake in *Neurospora crassa*. J. Gen. Microbiol. **13**: 72–83.

MOYED, H. S., and B. MAGASANIK, 1960 The biosynthesis of the imidazole ring of histidine. J. Biol. Chem. **235**: 149–153.

PAULY, H., 1904 Uber die Konstitution des Histidins. Z. Physiol. Chem. **42**: 508–518.

SUTHERLAND, E. W., C. F. CORI, R. HAYNES, and N. S. OLSEN, 1949 Purification of the hyperglycemic-glycogenolytic factor from insulin and from gastric mucosa. J. Biol. Chem. **180**: 825–837.

WEBBER, B. B., and M. E. CASE, 1960 Genetical and biochemical studies of histidine requiring mutants of *Neurospora crassa*. I. Classification of mutants and characterization of mutant groups. Genetics **45**: 1605–1615.

THE SPECTRUM OF SEVERITY OF MUTANT EFFECTS. II. HETEROZYGOUS EFFECTS IN YEAST

ALLEN P. JAMES

Atomic Energy of Canada Limited, Chalk River, Ontario

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ALTHOUGH dominance is a much investigated phenomenon, more attention has been given to the qualitative aspects of particular allelic interactions than has been directed towards outlining any over-all patterns. Recently, questions concerning the genetic hazards of radiation to populations have emphasized the need for more precise quantitative information on this subject. Dominance is one of the factors that must be considered in predicting the fate of a mutant gene in a population. Yet direct quantitative information on the partial dominance of recessive lethal mutations is confined to a very few studies of which the comprehensive investigation of STERN, CARSON, KINST, NOVITSKI and UPHOFF (1952) is outstanding. Direct information on the dominance of nonlethal mutations is even more scant.

Of particular interest are mutant genes which have a selective advantage in the heterozygote; in this case mutation can have little effect on gene frequency. At present there is considerable uncertainty about the frequency with which mutant genes display a heterozygous advantage. Studies by WALLACE (1957) and by BURDICK and MUKAI (1958) have indicated that increased viability resulting from increased numbers of heterozygous genes may be common in Drosophila under some circumstances. On the other hand, in studies with mice (RUSSELL 1957) no evidence has been found of an increased life span in the progeny of irradiated mice. The conflicting results of such experiments have yet to be explained.

The present investigation was designed to provide quantitative information on the dominance of mutant genes in yeast. Radiation-induced and spontaneous mutations affecting a quantitative character, rate of growth, were studied. Three aspects of the problem received particular attention: (1) the mean effect of negative mutations in the heterozygous condition, (2) the frequency with which mutant genes display a heterozygous advantage, and (3) the relationship between degree of dominance and extent of mutant effect in the haploid.

TECHNIQUES AND PROCEDURES

The techniques used to estimate the heterozygous effects of mutant genes were very similar to those used by the author in detecting mutations in yeast, and in estimating their haploid effects (JAMES 1959; JAMES and SPENCER 1958). Detailed descriptions of procedures will therefore be omitted from the present report except where techniques differ from those of the publications cited above.

General procedure: Rates of growth were expressed in terms of the "one point growth values" of the different strains. These values were the logarithms of the diameters of microcolonies on solid medium, divided by the number of hours (usually about 48) elapsing since the initial inoculation of individual budding cells. Although the values obtained are not free of minor biases, the technique can be used successfully for detecting and estimating the extent of very small differences in rate of cell division.

All the mutant genes under study were detected in a single diploid strain of *Saccharomyces cerevisiae* (strain 4819) that had been developed in this laboratory. This strain had been inbred for 27 generations and was considered to be essentially homozygous. The majority of the mutations were obtained in a single investigation already reported (JAMES 1959). To these were added a few subsidiary mutations detected by the same technique in two preliminary investigations.

The mutants were maintained in the heterozygous condition as clones. Each clone consisted of the vegetative descendants of a single cell isolated from a colony produced by the plating of either irradiated or unirradiated cells of a population of strain 4819. The cells of this latter population were themselves the immediate descendants of a single cell.

To determine the effect of a mutant gene on the growth rate of a heterozygote, the growth rate of the heterozygote was compared with that of one or more clones that were apparently free of mutant genes and that had been derived from the same inbred, though unirradiated, population. To estimate the dominance of a mutant gene, the effect of the gene on the haploid was compared with its effect on the heterozygote. Dominance was defined as that fraction of the haploid effect which was displayed by the heterozygote. A heterozygous advantage was considered to be held by those mutants which displayed overdominance of mutant genes; the heterozygote grew more rapidly than either homozygous normal diploid or mutant haploid.

Sixteen hours prior to testing, the cells of a clone were transferred from refrigerated broth culture to a presporulation medium and incubated at 33.5°C. The purpose of this procedure was to minimize any physiological differences between cells of a test clone or between cells of different test clones. At the same time, the procedure tended to equalize the number of generations preceding the estimation of heterozygous effects and the number preceding the estimation of haploid effects.

In most of the experiments, two budding test cells and two budding control cells were transferred to four marked locations on fortified agar droplets. These were incubated in Van Tieghem cells at 21.5°C. Each test clone was usually represented in five Van Tieghem cells. The data from a day's isolations formed a "lot". In the case of subsidiary mutations mentioned earlier, each Van Tieghem cell contained only two isolations, both representative of the same clone.

Statistical analysis: The growth values of any one clone, whether test or control, are likely to include values that are obviously atypical. Some of these are probably a result of experimental error. Others are no doubt attributable to

genetic heterogeneity within a clone. Here the most common culprits are respiratory-deficient mutants; the growth rate of these variants is relatively slow. But whatever the origin of the atypical values, efficient analysis of the data demands their exclusion. Although respiratory deficiency can be detected with assurance by spectroscopic methods, individual colonies were not tested for this character because of the amount of labor involved. Rather, atypical values (or many of them) were excluded through the use of a rejection criterion based upon the range (BLISS, COCHRAN and TUKEY 1956). In this system the ratio of the largest difference between paired values to the sum of all differences is used to determine whether there is a statistically significant amount of heterogeneity. Values are rejected sequentially. The method did not discriminate between the two members of a pair, but it was so conservative in its rejections that there was at no time any doubt as to which of two values was discordant in this investigation. This was invariably the lower. On occasion a value was unpaired due to failure to isolate a viable cell. More rarely, inspection of the data suggested that both members of a pair of colonies were atypical of the clone. In either of these instances values were excluded if they were lower than the highest value consistently excluded by the statistical test.

The statistical significance of a difference between mean growth rates of a test and of a control clone was determined by a test of the null hypothesis as applied to the differences between paired values (first control isolate *vs.* first test isolate, and second control *vs.* second test isolate). The growth rate of a clone was defined in terms of its performance comparative to that of its control; here, heterozygous effect is the mean growth value of a test clone divided by the mean growth value of its control.

For subsidiary data, a t test involving group comparisons was used. The growth values of all control clones not identified as mutant served as a control.

RESULTS

Dominance was studied in 75 clones that were heterozygous for mutations affecting rate of cell division. The haploid phenotypic effects of these mutations ranged from lethal to positive and included some that differed from normal by as little as 3.5 percent (JAMES 1959).

Some of the mutations were of induced and some were of spontaneous origin. All those that had been found in nonirradiated cells were, by definition, of spontaneous origin; those that had been found in irradiated cells must have included both induced and spontaneous mutations.

It had been intended that this investigation obtain information on induced mutations as distinct from spontaneous mutations. This was not because of any idea that there was a basic difference between mutations of the two origins, but only because it was expected that the spontaneous mutations present in the control population would not be representative of spontaneous mutations in general. It was intended that information on spontaneous mutations be used only to "correct" the sample of mutations found among irradiated cells. As it turned out,

such a correction was feasible with positive mutations but not with negative mutations. The number of negative mutants among the control clones was by no means negligible, but there was little evidence of a substantial frequency of any one type. Further, it was evident that some of these mutants were not represented within the class of irradiated clones. Under these circumstances, the removal of spontaneous mutations from the class of induced mutations was not justified. Consequently, all negative mutations were considered as a single group with the realization that the distribution might well be somewhat biased.

For simplicity in presenting results, dominance within the group of recessive lethal and near-lethal mutations will be considered separately from that within the group of nonlethal mutations.

Recessive lethals and near-lethals

Thirty-five clones, all of them known to be heterozygous for recessive lethals or near-lethals, were assayed for rates of cell division. The summarized results of these tests are presented in Tables 1 and 2. It is evident that the effects of

TABLE 1

The relative growth rates of heterozygotes for lethals and near-lethals

Test clone	Mutant type†	No. cells isolated	No. values rejected	Control clone	No. cells isolated	No. values rejected	Hetero-zygous effect	Statistical analysis		
								No. comparisons	t	Sig.
C–22	simple	9	0	C–17a	10	0	1.059	9	4.8	**
I–2	simple	10	0	C–2	10	0	1.066	10	2.6	*
–6	simple	6	0	C–3	10	0	1.128	6	11.5	**
–7	simple	10	0	C–5	10	1	1.019	9	0.7	..
–18	simple	8	0	C–13	9	0	.974	7	1.4	..
–23	simple	9	1	C–15	10	1	1.084	7	3.5	*
–29	simple	9	1	C–19	10	1	.972	8	1.5	..
–30	simple	10	2	C–20	10	0	.967	8	1.4	..
–32	simple	9	1	C–24	10	0	1.103	8	3.4	*
–52	simple	10	0	C–2a	10	0	1.015	10	0.7	..
–53	simple	10	2	C–3a	10	0	1.036	8	2.9	*
–54	complex	10	0	C–5a	10	0	.997	10	0.2	..
–56	simple	10	0	C–7a	10	3	.895	7	5.2	**
–59	simple	10	0	C–12a	10	0	.961	10	2.2	*
–60	complex	9	2	C–13a	10	0	.919	7	9.1	**
–61	complex	7	0	C–17a	6	0	.652	6	19.8	**
–63	simple	7	0	C–14a	8	0	.732	7	20.0	**
–66	simple	6	0	C–17a	6	1	1.019	5	1.0	..
–69	simple	10	1	C–17a	9	0	.997	8	0.3	..
–76	simple	10	0	C–17a	10	0	.594	10	17.7	**
–83	simple	10	1	C–17a	9	0	1.060	8	1.6	..
–89	complex	10	8	C–17a	10	1	1.159	2	2.9	‡
–93	simple	10	1	C–17a	10	0	.998	9	0.1	..

* Significant at 5 percent level.
** Significant at 1 percent level.
† "Simple" mutants segregated 2:2 for the detrimental mutation, whereas "complex" mutants produced one or more asci that contained more than two affected segregants.
‡ The number of rejected values indicated that this strain was an unstable "fast" grower.

TABLE 2

The relative growth rates of heterozygotes for subsidiary lethals and near-lethals

Test clone	Haploids		Heterozygotes				
	Mutant type†	No. segregations analysed	No. cells isolated	No. values rejected	t	Sig.	Heterozygous effect
C–208	simple	2	6	0	17.4	**	.691
–209	simple	2	8	0	0.4	..	.995
I–201	simple	5	9	0	9.6	**	.831
–203	complex	2	9	0	4.8	**	.941
–204	complex	4	6	0	0.8	..	.989
–207	complex	4	7	0	11.9	**	.833
–218	complex	4	8	0	2.0	*	.966
–219	simple	1	9	0	5.2	**	.913
–220	simple	4	10	0	3.9	**	.934
–221	simple	4	10	0	4.0	**	.934
–303	simple	9	8	0	3.7	**	.943
–316	simple	4	10	0	0.2	..	.997

* Significant at 5 percent level.
** Significant at 1 percent level.
† "Simple" mutants segregated 2:2 for the detrimental mutation, whereas "complex" mutants produced one or more asci that contained more than two affected segregants.

mutant genes on growth rate are extremely diverse; the estimated changes range from a 40 percent reduction to a 16 percent increase in rate of growth. Moreover, the differences between test clones and control clones are statistically significant in over 60 percent of the comparisons. In two thirds of these the test clones grew more slowly; in one third they grew more rapidly.

Differences fell short of statistical significance in only 13 of the heterozygotes. And here, the absence of a significant effect could not be construed as evidence that the mutant genes in question were completely recessive. Rather, it was an indication only that any mutant effects in these heterozygotes were probably small. The growth rates, as measured, indicated that nine were lower and four higher than those of the respective control clones.

Despite the frequency with which dominance was displayed, the modal value of these data is reduced from that of the controls by less than two percent. The mean value is reduced by about five percent.

Of major interest is the initial indication that overdominance of lethal or near-lethal mutations may be frequent. Thirty-one percent of the test clones grew faster than their respective control clones; in 20 percent (7 of 35) the differences in rates were statistically significant. To the above list might perhaps be added some instances in which overdominance was present but masked in the data for one reason or another. An upper limit to the estimate of frequency of overdominance of mutant genes is thus an amount somewhat in excess of 30 percent.

On the other hand, the data do not contradict the possibility that the actual frequency with which mutant genes display overdominance in this sample is very low if not zero. In some instances, indications of overdominance may be, in reality, a result of sampling errors. Even the demonstration that a test clone grew

significantly faster than its control does not by any means provide conclusive evidence that overdominance is the cause of its behavior; and where the increased rates are correctly attributable to overdominance, the frequency of the clones in question may have been so affected by selection pressures as to be unrepresentative of the true frequency with which recessive lethals and near-lethals are overdominant. The data thus indicate only that the frequency is somewhere between zero and one third. Subsequent investigations of these clones were concerned with improving this estimate. Here, no attempt was made to provide indisputable evidence that a particular gene did or did not display overdominance. Rather, clones were screened for those instances in which there was good evidence that some mechanism other than overdominance was responsible for clonal behavior.

An important circumstance, other than sampling or experimental errors, that can lead to faulty estimates of dominance or overdominance is the unrecognized presence of a mutant gene within a clone. The extent and direction of the resulting error in estimate and the ease of detecting that error are dependent on such factors as whether the extraneous mutation is positive or negative in its effect on the heterozygote, whether it is of induced or spontaneous origin, whether it occurs in a test or control population, and whether the affected population is homogeneous or heterogeneous for it.

Ignoring for the time being the possible frequency with which overdominance may be masked, conditions likely to produce spurious evidence of overdominance will be considered. Three such conditions are those in which (1) test clones are homogeneous for induced or spontaneous dominant positive extraneous mutations, (2) control clones are homogeneous for spontaneous dominant negative mutations, and in which (3) test clones are heterogeneous for spontaneous dominant positive mutations. The following sections are concerned with determining whether any of the above circumstances prevailed in those instances where initial results intimated the existence of overdominance.

Homogeneity of test clones for dominant positive extraneous mutations: When a test clone is homogeneous for a positive mutation, that mutation may have been either spontaneous or induced, but it must have been present prior to the latest single cell isolation in the history of the clone. Thus it must have been present in all cells at the time the clone underwent segregation analysis. That such mutations should sometimes go undetected in that analysis is not surprising in view of the fact that the experimental method was not designed to detect small-effect mutations in the presence of large-effect mutations (JAMES 1959). In fact, previous investigations of the test clones had made no attempt to detect either positive or negative mutations in clones known to be heterozygous for lethals or near-lethals. It had been considered that the excessively slow growth, if not the absence, of two or more segregants from each ascus rendered detection of non-lethal mutations, even of large effect, too unlikely to be worth attempting. Nevertheless, it was found that, in all likelihood, increased growth rate of some of these test clones was attributable to the presence of positive mutations.

In Figure 1 the growth values of the "normal" segregants of heterozygotes for lethals and near-lethals have been arranged in a frequency distribution. Of the

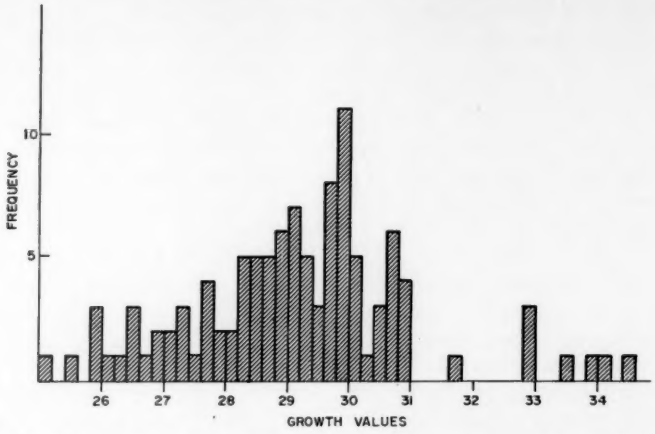

FIGURE 1.—The frequency distribution of the growth values of "normal" segregants of diploids heterozygous for recessive lethals or near-lethals.

eight highest values, seven refer to segregants of one or other of the four fastest growing heterozygotes. One stems from I-6, three from I-23, one from I-32 and two from I-89. There can be little doubt that this association of rapidly reproducing segregants with rapidly reproducing heterozygotes is more than coincidence, and that the increased rate of cell division in the four clones is attributable, not to overdominance of negative mutant genes but to the simultaneous presence of dominant positive mutant genes. It should be noted that one of the eight highest growth values was produced by I-63, a clone that obviously did not have an excessively fast growth rate. Here it must be assumed either that (1) the clone was heterozygous for a positive mutant gene that failed to raise the rate of growth above normal, or that (2) the fast growing segregant was a rare mutant of spontaneous origin.

The above screening technique thus reduced the number of clones in which the presence of overdominance is intimated from 11 to seven.

Dominant negative mutants among control clones: Because they are at a selective disadavantage, the influence of spontaneous dominant negative mutations must be low. However, it is not impossible for such mutations to become fixed in a clone through the process of single cell isolation. It thus seemed quite possible that some of the clones used as controls were homogeneous for previously undetected mutations that were negative in the heterozygote; certainly the fact that mutations were detected by segregation analyses (JAMES 1959) invited the suspicion that still others remained undetected. A comparison of the growth rates of different controls within a lot provided an opportunity to detect such mutant control clones along with those controls that had low values by virtue of sampling errors. The mean growth value of a suspected mutant clone was compared, by a t test, with the remainder of the controls within a lot. A suspect was selected for

test on the basis of its growth relative to that of the test clone with which it had been paired and not on the basis of its growth relative to the other control clones within a lot. The most obvious suspects were the control clones against which the remaining "significantly fast" mutants—C-22, I-53 and I-2—were tested. These were C-17a, C-3a, and C-2. Of these, C-17a gave little evidence of being negative, and it was not a slow grower in later tests. But C-2 had a growth rate that was lower than that of the remainder of the clones in the lot in which it belonged, and C-3a ranked second lowest of the clones in its lot. A statistical analysis, however, failed to impute significance to the differences.

Heterogeneity of test clones for dominant positive mutations: Genetic heterogeneity within a test clone must be the result of spontaneous mutation. The fact that heterogeneity was not uncommon had already been suggested in a study of the haploid segregants of the test and control clones (JAMES 1959). Such spontaneous mutations, when positive and dominant, make the accurate estimation of dominance particularly hazardous. Combined with the fact that they can easily escape detection is the fact that selection pressure for such mutants must be very strong. Here it should be noted that the process by which outliers were rejected from the data was far too conservative to reject other than negative, and presumably respiratory-deficient, variants.

An efficient method of testing for genetic heterogeneity within a clone is to determine whether or not the difference between the lowest and the highest growth values produced by a sample of cells is attributable at least in part to hereditary differences.

This may be accomplished by isolating and replanting cells from each of the two colonies that provided these high and low values. These cells themselves provide two new sets of growth values which may be compared; a statistically significant difference between the means of the two sets of values implies an hereditary difference between the two colonies that were sampled. This, in turn, indicates the presence of genetic heterogeneity within the original clone.

When heterogeneity is detected by the above method, there must be some doubt as to which of the two types is the spontaneous mutant. The faster of the two was so designated on the grounds that it is not likely that a spontaneous mutation with a selective disadvantage would attain a frequency high enough to make detection likely unless the mutation rate was extremely high.

Although heterogeneity must sometimes remain undetected by the above procedure, failure to demonstrate significant differences within clones suggests homogeneity. As a consequence, the new information on growth rate of the clone under test may be pooled with the original information to obtain what is likely to be a more accurate estimate of growth rate.

Heterogeneity tests as described above were carried out with 11 of the clones listed in Table 1. These clones were the ones whose estimated growth rates had not differed significantly from control or in which positive effects had not been attributed to some factor other than overdominance (exceptions were C-22 and I-7 which were not included, and I-53 which was).

Summarized results are presented in Table 3. Heterogeneity was demonstrated

TABLE 3

Genetic heterogeneity within test clones heterozygous for lethals and near-lethals

Test clone	Original estimate of heterozygous effect	Reisolate "fast" or "slow"	Heterozygous effect	No. cells isolated	No. values rejected	Control clone	No. cells isolated	No. values rejected	No. comparisons	t (f *vs.* s)	Revised estimates		
											Entries	t	Heterozygous effect
I–2	1.066*	f	.959	8	0	C–17a	8	2	6	0.6‡	f+s	3.1**	.962
		s	.964	10	0	C–17a	10	1	9	..			
–7	1.019	†	.971	10	1	C–17a	9	2	7	..	orig.+ resample	0.4	.999
–18	.974	f	1.061	10	2	C–17a	10	0	8	4.2**	s	4.3**	.894
		s	.894	10	2	C–17a	10	0	8	..			
–29	.972	f	.940	9	1	C–17a	10	0	8	2.1	orig.+	4.2**	.964
		s	.976	10	0	C–17a	10	0	10	..	f+s		
–30	.967	f	.982	10	1	C–17a	10	1	9	1.1	orig.+	0.4	.987
		s	1.010	10	0	C–17a	10	0	10	..	f+s		
–52	1.015	f	1.049	10	5	C–17a	9	0	5	3.7**	s	3.9**	.944
		s	.944	8	0	C–17a	8	0	8	..			
–53	1.036*	f	.986	9	1	C–17a	9	0	7	0.6‡	f+s	0.2	.995
		s	1.002	10	0	C–17a	10	0	10	..			
–54	.997	f	.999	9	0	C–17a	10	0	9	0.9	orig.+	0.8	.992
		s	.980	10	1	C–17a	10	0	9	..	f+s		
–66	1.019	f	.993	10	0	C–17a	9	0	9	0.1	orig.+	0.2	.999
		s	.993	10	1	C–17a	10	0	10	..	f+s		
–69	.997	f	.958	10	0	C–17a	10	0	10	1.5	orig.+	3.3**	.976
		s	.980	10	1	C–17a	10	1	8	..	f+s		
–83	1.060	f	1.100	10	0	C–12a	10	0	10	3.7**	s	0.1	.997
		s	.997	10	1	C–12a	10	1	9	..			
–93	.998	f	1.004	10	0	C–12a	10	0	10	0.5	orig.+	0.2	.998
		s	.989	10	0	C–12a	8	1	7	..	f+s		

* Significant at 5 percent level.
** Significant at 1 percent level.
† Original clonal culture was resampled.
‡ Reisolates did not differ significantly from each other but did differ significantly from the original.

in three of the clones. These were I-18, I-52, and I-83. For one of these, I-18, a nonsignificant decrease in growth rate was re-estimated as significant. For one, I-52, a nonsignificant increase was revised to a significant decrease, and for one, I-83, a nonsignificant increase was revised to a nonsignificant decrease. It may be noted that in each of the above instances, the original estimate conforms to expectation by being intermediate in value between the two values obtained subsequently.

It is of interest that the previous intimation that the "overdominance" of I-2 and I-53 was attributable to low mean control values was upheld by the heterogeneity tests: reisolates of I-2 did not differ from each other but did differ significantly from the original. The same was true of I-53.

In the last column of Table 3 are the revised estimates of heterozygous effects of the mutant genes. These are the lower of the two reisolate values where heterogeneity was detected and the mean of the three values (original plus two reisolates) when heterogeneity was not indicated. That the revised values are in fact more accurate estimates depends not only on whether heterogeneity or its absence was correctly assigned, but also on whether the two control clones C-17a and C-12a were nonmutant. Evidence on this point was obtained by determining if test clones or their homogeneous reisolates gave the same mean phenotypic effect when tested against C-17a or C-12a as when tested against an assortment of control clones. Seven test clones had a mean relative growth value of 1.007 when tested against seven different controls and 1.015 when tested against C-17a. Twelve clones produced a mean value of .994 as compared with .985 in a similar test of C-12a. It is not likely that the two clones were mutant.

In Figure 2 the final estimates of heterozygous effects of recessive lethals and near-lethals are shown in histogram form. Three facts are noteworthy: (1) The

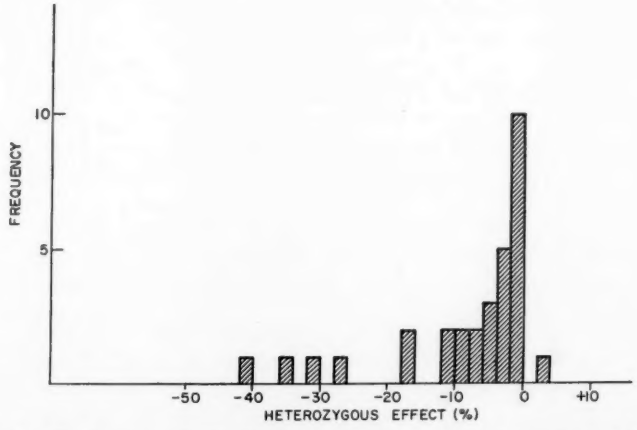

FIGURE 2.—The frequency distribution, by heterozygous effects, of recessive lethal and near-lethal mutations.

modal value is lower than that of the homozygous normal by an amount between zero and two percent. (2) There is a mean reduction of 8.1 percent in rate of growth. (Six of these clones produced segregations that indicated some complexity of mutant effect; their asci sometimes contained more than two affected spores. If these complex mutants are eliminated from the data, the mean reduction in growth rate is 8.6 percent.) (3) There is no evidence of overdominance among X-ray-induced lethal or near-lethal mutations. Only the control clone, C-22, remains as a candidate for overdominance, and it is not a very strong one since it was not tested for genetic heterogeneity.

Positive mutations

Six clones known to be heterozygous for mutations that had a positive effect on growth rate of the haploid were available for test. As two of these were controls and all of them had similar phenotypes, it seemed quite likely that some if not all these positive mutations had a common spontaneous origin. This contention was not contradicted in a comparison of effects in the heterozygote; something approaching full dominance was displayed by all but one of the mutants (Table 4). The exception was I-67 with which a significant degree of dominance became evident only with further testing. Since there was considerable evidence that this clone was in reality a dihybrid, the low degree of dominance may well be attributed to epistasis of the negative mutant.

It is noteworthy that the fast-growing heterozygotes tended to be unstable producers of respiratory-deficient cells (see frequency of rejected values in Tables 1 to 6), a circumstance expected when cells have an excessively rapid rate of cell division (JAMES and SPENCER 1958). It is likely that rate of growth is underestimated in these unstable clones because each colony contains a high concentration of slowly dividing variants.

Nonlethal negative mutations

Dominance of nonlethal negative mutations was studied in 34 clones. The results of initial tests are shown in Tables 4 and 5. As measured, 17 of the clones grew faster, and 17 grew more slowly than did their respective controls. The modal value was close to normal; the mean value was 1.002. But any implication of complete recessiveness on the part of the mutant genes was discounted by the fact that the rates of six of the test clones differed significantly from those of their controls. Four were higher and two were lower.

Again, as with lethals and near-lethals, a degree of overdominance is suggested. This can hardly exceed 50 percent—the proportion of clones that grew faster than their controls—and it may be as low as zero.

The uncertainty attached to the estimate of overdominance among detrimentals was reduced by the same procedure as was used for lethals and near-lethals, with the exception that a search was not made for previously undetected positive segregants in clones known to contain negative mutants. With these clones it was not possible to carry out a more thorough search than had already been undertaken (JAMES 1959). Certainly, none of the four "fast" heterozygotes had, in their

TABLE 4

The relative growth rates of heterozygotes for nonlethal mutations

Test clone	Haploid effect	No. cells isolated	No. values rejected	Control clone	No. cells isolated	No. values rejected	Hetero-zygous effect	Statistical analysis		
								No. comparisons	t	Sig.
C–4	.863	10	1	C–17a	10	0	.992	9	0.4	..
–8	1.111	10	1	C–17a	10	0	1.118	9	6.0	**
–10	.868	10	2	C–17a	10	1	1.019	8	0.8	..
–23	1.092	10	6	C–17a	10	0	1.125	4	5.1	*
–25	.914	10	1	C–17a	10	1	.992	9	0.4	..
–27	.899	13	0	C–12a	14	0	.998	13	0.2	..
I–8	1.102	8	2	C–6	7	1	1.083	4	3.4	*
–9	.387	10	0	C–17a	9	1	.997	8	0.3	..
–11	.965	10	0	C–9	10	1	1.013	9	0.7	..
–13	1.094	9	0	C–11	10	0	1.096	9	4.6	**
–15	.965	10	0	C–12	10	0	.986	10	1.2	..
–24	.899	10	0	C–16	10	0	1.025	10	0.5	..
–26	.962	9	1	C–17	9	2	.960	5	1.1	..
–31	.690	10	1	C–21	10	0	.990	9	0.5	..
–40	.956	10	2	C–18	9	1	1.040	8	3.3	*
–41	1.181	10	4	C–26	9	0	1.064	6	3.6	*
–43	.882	10	2	C–28	10	0	.984	8	0.5	..
–44	.953	27	10	C–12a	27	0	1.015	17	1.7	..
–45	.955	10	2	C–29	10	0	1.054	8	2.6	*
–46	.873	10	0	C–30	10	0	1.009	10	0.6	..
–47	.965	9	0	C–31	10	1	.988	8	1.3	..
–48	.781	10	0	C–32	10	0	1.007	10	1.7	..
–50	.964	10	0	C–39	10	0	1.013	10	0.8	..
–51	.796	10	0	C–1a	10	0	1.100	10	5.3	**
–55	.959	10	1	C–6	10	2	.973	8	2.2	..
–58	.890	10	2	C–11a	10	0	.948	8	3.1	*
–67	1.135–.797	8	0	C–17a	8	0	1.010	8	1.5	..
–78	.798	10	0	C–17a	10	1	1.008	9	0.6	..
–79	.952	8	0	C–17a	8	0	.997	8	0.2	..
–80	.554	8	0	C–17a	8	0	.991	8	0.5	..
–84	.822	10	0	C–17a	9	0	.964	9	3.5	**
–91	.941	10	5	C–17a	10	0	1.036	5	3.1	*
–92	.765	9	0	C–17a	10	1	1.017	8	0.9	..
–94	.871	9	3	C–17a	10	2	1.010	6	0.5	..

* Significant at 5 percent level.
** Significant at 1 percent level.

segregations, given any indication that they contained positive mutations.

Negative mutants among control clones: Statistically significant differences between rates of growth of test clones and their controls had indicated overdominance in four test clones, I-40, I-45, I-51, and I-91. The four corresponding control clones were scanned for evidence that the differences were in fact due to reduced rates of growth of these controls. They were C-18, C-29, C-1a, and C-17a. Of these, C-17a had already been classified as a probable nonmutant, and C-29

had only the third lowest mean growth value of eight clones in one lot. But C-18 had the second lowest mean growth value of eight and C-1a had the lowest of eight. A t test showed the growth values of C-1a to be significantly lower than those of the remaining control ($P = < .01$). Thus, there is good evidence that the mutant tested against C-1a was not, in fact, overdominant.

Genetic heterogeneity of test clones: Heterogeneity tests (similar to those already described) were confined to 17 test clones, but among these were the four clones in which overdominance had been indicated. Results are summarized in Table 6. Genetic heterogeneity was demonstrated in three clones, I-24, I-46, and I-79. In two of these a significant negative effect was unmasked. Genetic heterogeneity could not be attributed to any of the four "overdominant" clones. But the test did confirm the previous evidence that the growth rates of the control clones C-1a and C-18, as measured, were extraordinarily low; the combined data from paired reisolates differed significantly from the original isolates I-40 and I-51. Both clones could be removed as candidates for overdominance.

With the compilation of re-estimates the number of clones having rates of growth exceeding that of their controls was reduced from 17 to 13. The number of clones showing a significant increase attributable to overdominance stood at two. These were I-45 and I-91. But the data of Table 6 do not remove the suspicion that genetic heterogeneity existed in these two clones despite the absence of statistically significant evidence of this.

The distribution of heterozygous effects of nonlethal mutations, as finally estimated, is shown in Figure 3. The modal value is reduced somewhat, and the mean is reduced by 0.6 percent.

Considering all the detrimental mutations, whether lethal or nonlethal, the mean rate of growth is reduced by an estimated 4.2 percent. If the calculation of the mutant effect is refined somewhat by omitting complex mutants which may not be representative of mutations in general and by omitting those mutants in which overdominance is implied by a significant alteration in rate, the reduction is 4.4. percent, a value that is not very different.

Data of this paper together with that of the preceding paper (JAMES 1959) provide the information necessary to determine whether there is correlation

TABLE 5

The relative growth rates of heterozygotes for subsidiary nonlethal mutations

Test clone	Haploids		Heterozygotes				
	Effect	Stat. sig.	No. cells isolated	No. colonies rejected	Effect	t	Stat. sig.
C–201	.900	**	9	0	.931	3.8	**
–203	.923	**	9	0	.971	1.7	..
I–208	.637	**	8	0	.989	0.6	..
–209	.640	**	10	0	1.016	0.9	..
–210	.403	**	9	0	1.022	1.9	..
–222	.802	**	10	0	1.029	1.9	..

** Significant at 1 percent level.

TABLE 6

Genetic heterogeneity within clones heterozygous for nonlethal mutations

Test clone	Original estimate of heterozygous effect	Reisolate "fast" or "slow"	Heterozygous effect	No. cells isolated	No. values rejected	Control clone	No. cells isolated	No. values rejected	No. comparisons	t (f *vs.* s)	Revised estimates: Entries	t	Heterozygous effect
C–4	.992	f	.988	10	1	C–12a	10	0	9	0.5	orig.+	0.6	.993
		s	1.001	10	1	C–12a	10	0	9	..	f+s		
–10	1.019	f	.997	10	0	C–12a	9	1	8	0.2	orig.+	0.5	1.007
		s	1.005	10	0	C–12a	9	3	6	..	f+s		
–25	.992	†	1.000	17	1	C–12a	17	1	14	..	orig.+ resample	0.3	.997
I–8	1.083*	f	1.075	10	2	C–17a	10	0	8	1.2	orig.+	10.6**	1.087
		s	1.098	10	0	C–17a	10	0	10	..	f+s		
–15	.986	f	1.030	10	1	C–12a	9	0	8	0.9	orig.+	0.7	1.006
		s	1.009	8	0	C–12a	7	0	7	..	f+s		
–24	1.025	f	.938	9	0	C–12a	9	0	9	2.4*	f	2.9*	.938
		s	1.009	0	0	C–12a	10	0	9	..			
–26	.960	f	.972	10	0	C–12a	10	0	10	1.4	orig.+	2.1*	.980
		s	.997	10	0	C–12a	10	0	10	..	f+s		
–31	.990	f	.979	9	0	C–12a	10	1	9	0.4	orig.+	1.7	.979
		s	.968	10	0	C–12a	10	0	10	..	f+s		
–40	1.040*	f	1.008	9	0	C–12a	10	0	9	0.8‡	f+s	0.1	.998
		s	.986	8	0	C–12a	8	1	7	..			
–45	1.054*	f	1.002	8	0	C–12a	8	0	8	1.7	orig.+	2.7*	1.033
		s	1.046	10	4	C–12a	9	0	6	..	f+s		
I–46	1.009	f	1.057	10	0	C–12a	10	0	10	2.3*	s	0.4	.991
		s	.991	10	0	C–12a	10	0	10	.			

–47	.998	f	.965	10	1	C–12a	9	0	8	1.7	orig.+ f+s	1.7	.986
		s	1.006	10	1	C–12a	10	1	8				
–48	1.007	f	1.013	10	0	C–12a	10	0	10	1.0	orig.+ f+s	0.4	1.003
		s	.989	10	0	C–12a	10	0	10				
–51	1.100*	f	.983	10	0	C–12a	10	1	9	0.1‡	f+s	0.1	.999
		s	1.014	10	0	C–12a	10	0	10				
–55	.973	f	1.000	8	1	C–12a	8	1	6	1.7	orig.+ f+s	0.5	.973
		s	.957	10	1	C–12a	10	0	9				
–67	1.010	f	1.037	18	1	C–12a	18	2	15		orig.+ f	5.6**	1.027
–78	1.008	f	1.008	10	0	C–12a	10	0	10	0.2	orig.+ f+s	0.9	1.009
		s	1.012	10	0	C–12a	10	0	10				
–79	.997	f	1.081	10	0	C–12a	10	1	9	3.9**	s	2.3*	.972
		s	.972	14	1	C–12a	14	0	13				
–80	.991	s	.994	14	3	C–12a	14	1	11		orig.+ s	0.6	.993
–91	1.036*	f	1.005	10	0	C–12a	10	1	9	1.6	orig.+ f+s	2.8*	1.025
		s	1.040	10	0	C–12a	10	1	9				

* Significant at 5 percent level.
** Significant at 1 percent level.
† Original clone was resampled.
‡ Reisolates did not differ significantly from each other but did differ significantly from the original.

TABLE 7

The correlation of mutant effects in haploid and in heterozygote

Mutant type	No.	Haploid effect		Heterozygous effect				Dominance
		Range	Mean	Range	Mean	Sig.inc.	Sig. dec.	
Lethals and near-lethals	25	−100 to − 76%	−94.6%	−41 to +6%	−8.6%	1	16	−8.6/−94.6 = .09
Nonlethal, more negative	17	− 75 to − 12%	−26.9%	− 4 to +3%	0.0%	0	1	0/−26.9 = 0
Nonlethal, less negative	17	− 11 to −3.5%	− 6.1%	− 7 to +3%	−1.2%	2	5	−1.2/− 6.1 = .20

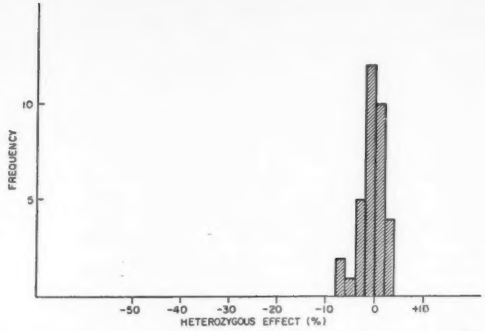

FIGURE 3.—The frequency distribution, by heterozygous effect, of nonlethal negative mutations.

between degree of dominance and mutant effect in the haploid. The individual heterozygous effects of all test mutants have been plotted against their haploid effects in Figure 4. The various degrees of a haploid effect are fairly well represented. The incidence of apparent overdominance is quite uniform over the entire range of haploid effects. But the degree of dominance is increased at the two extremes of the range. This is more obvious in the data of Table 7. Here mutant clones have been placed into one or other of three categories. One of these categories includes "simple" recessive lethals and near-lethals. The remaining 34 mutant clones have been divided equally into two categories, one including those that were more detrimental in the haploid, the other including those that were less detrimental. In the last column is the calculated mean dominance of the mutations in each category, where dominance is defined as that proportion of haploid mutant effect that is displayed in the heterozygote. There is a negative correlation between mutant effect in haploid and in heterozygote; the least detrimental mutations are the most dominant.

The reality of the correlation will be discussed later. Here it will be noted only that the correlation is not made suspect by the evidence that dominance is greater among lethals than among more detrimental nonlethal mutations. These lethals may well include a class of mutation that is unrepresented among nonlethals. For example, the presence of deletions involving several linked loci may be expected among lethals but would be rare among nonlethal mutations.

DISCUSSION

It can be expected that various factors act to bias the estimates of heterozygous effects obtained in this study. The extent and direction of these biases should be considered before any inferences are drawn from the data. They are of two general types (1) those that tend to make the list of mutant genes an unrepresentative samples of mutations, and (2) those that affect the dominance attributable to individual mutations.

The first part of this investigation (JAMES 1959) included a discussion of the

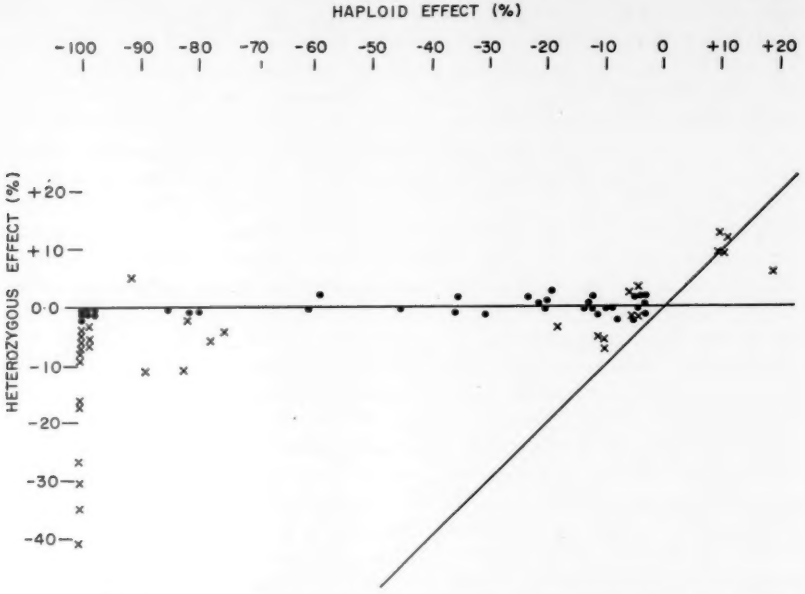

FIGURE 4.—The heterozygous and haploid effects of spontaneous and X-ray-induced mutations. Crosses indicate statistically significant heterozygous effects. Location of a point on the diagonal indicates complete dominance of the mutant gene. Location on the horizontal line indicates complete recessiveness. Location in the upper left sector indicates overdominance.

biases affecting the main sample of mutants. There it was concluded that the frequency of small effect mutations was probably underestimated. The present list includes all but five of these mutants. One of these, I-49, was positive, but the remaining four were small effect mutants in which growth rate was reduced by less than ten percent in the haploid. The shortage of small effect mutations is thus increased by these omissions. It is yet more aggravated through the addition of the subsidiary mutations; these were detected by a similar but less exacting procedure than were those of the main sample. Altogether, it seems certain that the frequency of minor mutations is considerably underestimated.

Several factors must tend to bias the estimate of individual heterozygous effects. One of these is the process by which outliers were rejected. It is evident that rejections were more rare in clones which were heterozygous for more negative mutations. As a result there was a tendency for negative mutant effects to be overestimated. But the error must be reduced where the effects of the mutations are less negative.

Another bias stems from the fact that although considerable effort was made to ensure that increased growth rate was not incorrectly ascribed to overdominance, a comparable effort was not made to demonstrate the possible or

likely existence of overdominance in those clones in which initial results did not indicate its existence. It was considered that the introduction of this bias was justified by the necessity for maintaining the amount of retesting at a minimum. Certainly, an attempt to demonstrate overdominance in clones which had significantly reduced growth rates in initial tests was too unlikely of success to be warranted. That the bias cannot be large is suggested by two facts. First, selective treatment was confined to those clones in which growth rate was significantly different from their controls. Second, overdominance must have been hidden only as a result of sampling error or coincidental pairing of an overdominant clone with a control containing an undetected dominant positive mutation. In this connection it may be noted that dominant positive mutations are less likely to go undetected in control clones than in test clones because in the former they are not masked by negative mutations. Altogether, the infrequency with which positive evidence of overdominance was detected indicates that the number of instances in which overdominance of equal or greater intensity was overlooked must have been low.

In considering these data it is important to remember that throughout the investigation selection pressure will have favoured spontaneous mutations with positive effects on rate of growth in the heterozygote. These positive effects could be due either to dominance of positive mutations or to overdominance of negative mutations. An attempt was made to minimize the effect of spontaneous mutations in biasing the data or in reducing its statistical significance, but it can have been only partially successful. For instance, each clone was derived from a single cell which was itself isolated from a colony that had been in existence for 20 or more cell generations. This procedure was followed in order to eliminate genetic heterogeneity due to such causes as delayed mutation and the plating of budding or binucleate cells. In retrospect it seems likely that the procedure was unwise. Considering the amount of genetic heterogeneity that was subsequently detected in test clones, the number of spontaneous mutations that were introduced and fixed in the clones must have been greatly increased by the procedure.

The estimated mean reduction in growth rate of heterozygotes for lethals and near-lethals, eight percent, is surprisingly similar to the reductions of four percent obtained by STERN *et al.* (1952), of 4.5 percent obtained by MULLER and CAMPBELL (MULLER 1950) and of seven percent obtained by CORDEIRO (1952) in viability of heterozygotes for lethals in Drosophila.

The mean reduction in growth rate, 4.2 percent, for all the negative mutations is obviously an overestimate of the true value because the frequency of minor mutations was underestimated. Inclusion of those mutations, presumably numerous, with haploid effects of less than 3.5 percent, would reduce the value even though there was a high degree of dominance among them.

There is a further consideration which suggests that to limit the sample to those mutants whose effects are greater than some arbitrary value may not be unjustified as a practical measure under certain circumstances. The estimate of mean mutant effect is needed to predict the effect of changes in mutation rate on a population. But a change in mutation rate will only be effective when the

mutation consistently confers a selective disadvantage. Otherwise, any change in gene frequency will be too small to be of consequence. It must be nearly certain that extremely negative mutant effects are consistently at a selective disadvantage. This is less certain in the case of small negative mutations. It is not unreasonable to suppose that in this portion of the spectrum of mutations, selection pressures tend to become inconsistent in an unstable environment, favouring a particular gene in some environments and in some gene complexes and favouring its allele in others. Here shifts in environment might be sufficient to maintain genetic heterogeneity, and mutation would be a relatively weak force.

The frequency with which detrimental mutant genes display a selective advantage in the heterozygote is of greater importance in this study than is the intensity of that advantage. A change in mutation rate will have little effect on gene frequency where a heterozygous selective advantage prevails even if the advantage is small or not always evident. In strain 4819 and with the conditions of growth under study here, the frequency with which detrimental mutant genes display a selective advantage in the heterozygote is obviously low. Only four test clones had growth rates that exceeded that of their controls by more than two percent, and these cases are examples of overdominance only by default. Although the increase in growth rate was significant in three of them, there was some reason for suspecting that overdominance was not the real cause.

The results of this investigation are in accord with those of RUSSELL (1957) and of FALK (1959) in indicating that radiation-induced mutations lower the mean value of a quantitative character. They are at variance with those obtained by WALLACE (1957) and by BURDICK and MUKAI (1958), where increases in mean viability following irradiation of homozygotes were found. This lack of accord between results is not surprising when different quantitative characters are being compared in different organisms. But it does weaken the confidence with which one can generalize from the results of any one investigation. It may be that there are basic genetic differences between the organisms; it has been suggested that in some of its features the genetic mechanism of Drosophila is atypical, and it might as well be suggested that yeast has atypical features. However, the fact that *S. cerevisiae* prefers to exist as a diploid although capable of existing as a haploid does imply that the organism's genetic make-up is conducive to overdominance. However, the divergence of results may be explained in any of several other ways that do not include basic genetic differences between the two species. Some of these will be considered below.

Growth rate may have been an unfortunate character from the point of view of uncovering evidence of heterozygous advantage in yeast. In this study overdominance has been equated with heterozygous advantage, but the two phenomena need not always be synonymous; the absence of overdominance does not ensure the absence of a heterozygous advantage. It seems likely that heterozygous advantage is frequently a result of the interaction of secondary effects of gene action. An example is provided by sickle-cell anemia. Here, recessiveness for anemia and dominance for resistance to malaria combine to produce overdominance for viability which is a heterozygous advantage. It is to be expected

that this type of complex interaction will increase in frequency as the effects of gene action are ramified. But for characters that more closely reflect primary gene action, overdominance may be more rare. We cannot easily locate growth rate on such a scale, but certainly it is only one of the factors that contribute to over-all fitness. On the other hand, hereditary changes in this character must be far removed from primary gene action in many instances.

Overdominance may be confined largely to mutations having little or no haploid effect on the character under consideration. Such mutations have been termed isoallelic. It would seem that genes in this category may have little that is basically in common; mutations with small effects on one character must frequently have large effects on some other character not under observation, and environment must frequently be the factor which controls whether or not a mutation is isoallelic. The present investigation provides no direct evidence on overdominance of isoallelic mutations since all the mutant genes under test had mutant effects exceeding three percent in the haploid. The investigation does, however, provide some indirect evidence against the hypothesis: (1) Isoallelic mutations must frequently have been present as undetected mutations in the test clones. If these were overdominant, one might expect the combination of the recessive mutation under test and the overdominant isoallelic mutation to produce evidence of overdominance. (2) Overdominance of isoallelic mutations is not consistent with the evidence that dominance increases with decreasing mutant effect. (3) Eight "irradiated" clones in which mutations had not been detected in segregation analyses were tested for growth rates. None differed significantly from its control, and the mean rate of growth was reduced by 0.5 percent.

Overdominance may have been frequent but individual intensities may have been too low for detection; or the phenomenon may have been masked by the presence of dominant negative mutations.

The gene complex of strain 4819 may have been inappropriate to the appearance of overdominance. It has been suggested by WALLACE (1957) that overdominance may not be usual in well-adapted homozygous lines. How well adapted the present strain is, it can only be conjectured. But that it was not reproducing at a maximum rate is clearly evident from the appearance of positive mutations. Also, an attempt to de-adapt the strain had been made by testing the clones at a temperature that was 12 degrees lower than any to which they had previously been subjected.

Altogether, it seems that although there are several possible reasons why the apparent low frequency of overdominance in this investigation may have been atypical, none of these is very persuasive.

The investigation has produced direct evidence that there is a negative correlation between mutant effect in the haploid, which is equivalent to the homozygote, and in the heterozygote.

At first glance this evidence is not particularly impressive. But the reality of the correlation becomes more apparent when it is realized that a low degree of dominance among highly detrimental mutations is much more easily detected

than a high degree of dominance among mutants that are only slightly negative in the haploid phase. A five percent reduction in mean growth rate of more negative clones would have been required to achieve the same degree of dominance as was measured in less negative clones. Such a reduction could be detected with ease. Yet a five percent reduction was not detected in any of the 17 heterozygotes. In fact, the mean reduction in growth rate of heterozygotes for more negative mutants was less than that in less negative mutants. The reality of the correlation is also indicated by the ratio of 5 to 1 for individually significant reductions in the two categories despite the absence of statistical significance. That the dominance among small effect mutations was a result of the presence of a cluster of identical spontaneous mutations is of course possible but most unlikely; the presence of such a group is entirely unexpected in view of the selective disadvantage that they would have. In fact, none of the detected mutant genes in control clones was other than recessive.

This negative correlation between mutant effect and dominance was predicted by MULLER (1950). It is based on the hypothesis that extremely detrimental mutations will, through time, accumulate a genetic background that will reduce their harmfulness in the heterozygote. Small-effect mutations, on the other hand, would probably not. The correlation has some bearing on the results to be expected from irradiating inbred lines. Because large-effect mutations, though detrimental, tend to be recessive, they would be initially masked, but small-effect mutations would be more immediately evident. If an inbred line was well adapted to its environment, most if not all the small-effect mutations would be detrimental. If, on the other hand, the strain was relatively unadapted, then a fraction, however small, of these mutations would be beneficial. It seems not impossible that discrepancies between the results of different investigations may be attributable to differences between strains in the frequency of dominant positive mutations of small effect. An experimental test of this hypothesis would not be easy to perform, since it requires the individual measurement of small homozygous mutant effects. Further, strains that were equally unadapted might differ in their response to irradition since they might well differ in number of genes contributing to the lower degree of adaptation. It may be noted that in none of the mouse or Drosophila investigations mentioned above was it technically possible to compare heterozygous mutant effect with homozygous mutant effect. Rather, it was necessary to compare the heterozygous effect with the homyozous normal and to infer the homozygous mutant effect. The present investigation perhaps provides negative support to the hypothesis: the absence of positive haploid mutations was accompanied by an absence or low frequency of positive effects in heterozygotes.

SUMMARY

Dominance in each of an array of mutant genes affecting a quantitative character was investigated in yeast. The character used was rate of growth. Mutations were of both X-ray-induced and spontaneous origin. Their effects on

the haploid varied from lethal to positive. The smallest measured effects were reductions of 3.5 percent.

The mean effect of recessive lethals and near-lethals on growth rate of heterozygotes was a reduction of eight percent. The modal value of these heterozygotes was within two percent of normal. The mean effect of nonlethal but negative mutations on heterozygotes was a reduction of 0.6 percent. The modal value approached normal.

There was little evidence of overdominance. Significant increases in rates of growth, interpretable in terms of overdominance but not assuredly such, were found in three of 69 heterozygotes for negative mutations.

Direct evidence of a negative correlation between mutant effect in the haploid (considered to be equivalent to the homozygote) and in the heterozygote was found; mean dominance was greater for mutations of small effect than for mutations of large effect.

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LITERATURE CITED

BLISS, C. I., W. G. COCHRAN, and J. W. TUKEY, 1956 A rejection criterion based upon the range. Biometrika **43**: 418–422.

BURDICK, A. B., and T. MUKAI, 1958 Experimental consideration of the genetic effect of low doses of irradiation on viability in *Drosophila melanogaster*. Proc. 2nd Intern. Conf. Peaceful Uses Atomic Energy **22**: 325–329.

CORDEIRO, A. R., 1952 Experiments on the effects in heterozygous condition of second chromosomes from natural populations of *Drosophila willistoni*. Proc. Natl. Acad. Sci. U.S. **38**: 471–478.

FALK, R., 1959 Viability of Drosophila heterozygous for irradiated chromosomes. Science **130**: 1416.

JAMES, A. P., 1959 The spectrum of severity of mutant effect. I. Haploid effects in yeast. Genetics **44**: 1309–1324.

JAMES, A. P., and P. E. SPENCER, 1958 The process of spontaneous extranuclear mutation in yeast. Genetics **43**: 317–331.

MULLER, H. J., 1950 Our load of mutations. Am. J. Human Genet. **2**: 111–176.

RUSSELL, W. L., 1957 Shortening of life in the offspring of male mice exposed to neutron radiation from an atomic bomb. Proc. Natl. Acad. Sci. U.S. **43**: 324–329.

STERN, C., G. CARSON, M. KINST, E. NOVITSKI, and D. UPHOFF, 1952 The viability of heterozygotes for lethals. Genetics **37**: 413–449.

WALLACE, B., 1957 The effect of heterozygosity for new mutations on viability in Drosophila: A preliminary report. Proc. Natl. Acad. Sci. U.S. **43**: 404–407.

SEX-LINKED RECESSIVE LETHALS IN DROSOPHILA WHOSE EXPRESSION IS SUPPRESSED BY THE Y CHROMOSOME

D. L. LINDSLEY, C. W. EDINGTON,[1] AND E. S. VON HALLE

Biology Division, Oak Ridge National Laboratory,[2] Oak Ridge, Tennessee

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THE expression of mutant phenotype is subject to genetically controlled suppression; in *Drosophila melanogaster* the presence of a Y chromosome may in certain instances eliminate or decrease the phenotypic consequences of an impairment of gene function. Two general modes of Y suppression may be postulated: the Y may perform the function of the altered gene or it may stimulate the genome to perform the same function or an alternative function whose end result is a return toward phenotypic normality. The first method is the way that any wild-type gene suppresses the expression of its recessive allele. An example of this type of action by the Y chromosome is seen in the case of the sex-linked recessive gene bobbed (*bb*). Normal alleles of *bb* are located in the chromocentral heterochromatin of the X and in the short arm of the Y chromosome; this sharing of loci is part of the evidence that these regions are homologous. Instances of lethal changes that are in the chromocentral heterochromatin and are therefore covered by the Y are known, but they are few and involve the *bb* locus (*Df(1)bb*, *Df(1)bb*^{1}, and *In(1)sc*^{4L,8R}). An example of the second type of Y action is seen in the suppression of variegated (V-type) position effects (GOWEN and GAY 1934), which, though a commonly observed phenomenon, is still not understood (for a discussion, see COOPER 1956). SCHULTZ 1941 has demonstrated the existence of lethal V-type position effects that can be made to survive by adding a Y to the genome. He showed that T(1;4)*w*$^{258-18}$, which survives as a homozygous female or as an X/Y male and is variegated for white, is inviable as an X/0 male and that X^{c} survives as a homozygous female or an X/Y male but not as an X/0 male. Similarly, he showed that *N*$^{264-6}$, *N*$^{264-9}$, and *N*$^{264-10}$, which are lethal as X/Y males, survive as X/Y/Y males (see BRIDGES and BREHME 1944).

It was our purpose in the experiments reported here to determine to what extent induced lethal changes are suppressed by a single Y chromosome and to determine which of the mentioned modes of suppression was operating in any recovered cases. Since the ordinary methods for detecting sex-linked lethals rely on expression of lethality in X/Y males, they overlook the class that we seek to demonstrate. The second aim was to accumulate a series of different chromocentral lethals to provide new material for further linear resolution of the proximal heterochromatin of the X chromosome. Finally, we hoped to gain

[1] Present address: Department of Biology, Florida State University, Tallahassee, Florida.

[2] Operated by Union Carbide Corporation for the United States Atomic Energy Commission.

some insight into the contribution of V-type position effect to X-ray-induced sex-linked recessive lethals.

In the first endeavor we were quite successful in finding that, at 3 and 4 kr, approximately one fifth (49/239) of all sex-linked recessive lethals are suppressed by a Y chromosome. In practically every case, these lethals were found to be associated with chromosome aberrations. It follows that the Muller-5 procedure overlooks an appreciable sample of lethals, preferentially aberration-associated lethals. Of the Y-suppressed lethals, however, only about one eighth (6/49) seem to be in the region that is homologous to the Y chromosome, and since all these are deficient for the same gene (i.e., the *bb* locus), it appears that we have not accumulated a very versatile array of proximal heterochromatic lethals. The remainder of the Y-suppressed lethals, i.e., 18 percent (43/239) of all sex-linked lethals, can be shown to be V-type position effects. To these can be added roughly another three percent that die as X/Y but not as X/Y/Y males to yield a minimum estimate of about 21 percent as the contribution of position-effect lethals to sex-linked lethals produced by 3 and 4 kr.

Methods for recovering Y-suppressed lethals

The initial attempts to recover Y-suppressed sex-linked recessive lethals consisted in simply crossing the F_1 females of an ordinary Muller-5 test ($= X^{*}/M\text{-}5$ where X^{*} designates the irradiated X, and M-5 designates $Ins(1)sc^{s1L,s8R}$, *S*, $sc^{s1+s8}w^{a}$ *B*) to XY/0 males (where XY represents an attached XY chromosome, specifically $Y^{s}X{\cdot}Y^{L}$, *Ins(1)EN*, dl-49, $y\ w\ v\ f$, following the terminology of LINDSLEY and NOVITSKI 1959) rather than M-5/Y males. In this way the irradiated X appeared in X/0 rather than X/Y sons; X^{*}/XY daughters from all lethal-bearing cultures were then testcrossed to M-5/Y males, and any producing X^{*}/Y sons were suspected of being X/0 lethal, but X/Y viable and were consequently retested. This method had the disadvantage of requiring subsequent crossing of every suspected lethal to check for X/Y viability. A crossing scheme was desired that would allow recovery of both X^{*}/Y and $X^{*}/0$ sons in the F_2 in such a way that their survival could be scored separately. E. NOVITSKI (personal communication) suggested that this might be accomplished by recovering the irradiated X in an F_1 female that was $X^{*}/M\text{-}5/Y$ in constitution and crossing to XY/0 males. Such females should produce six types of ova: M-5/X^{*}; Y; M-5; M-5/Y; X^{*}; and X^{*}/Y. The latter two types should occur with equal frequencies and, when fertilized by a nullo X, nullo Y sperm should produce $X^{*}/0 = y$ and $X^{*}/Y = y^{+}$ males, respectively, if X^{*} is marked with y and the Y is marked with y^{+}, i.e., $y^{+}Y$ ($= sc^{s}{\cdot}Y$ of MULLER 1948). Scoring the F_2 culture for survival of both, one, or neither type of males should allow classification of the irradiated X's into nonlethal, Y-suppressed lethal, or orthodox lethal, respectively. In actual practice, however, the incidence of secondary nondisjunction in the $X^{*}/M\text{-}5/Y$ females was so high, owing to the extreme sequential heterozygosity of the two X chromosomes, that a sample of X^{*}-bearing sons sufficiently large to allow unambiguous classification was seldom produced; consequently, many cultures had to be retested. However, more than half the Y-suppressed

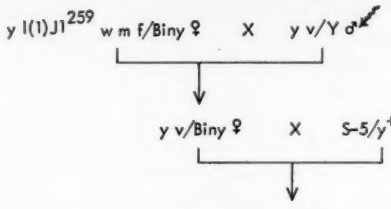
lethals described in this paper were recovered by this method. Owing to the ambiguity of the initial classification of cultures, this method was abandoned for one in which M-5/X° females could be used. The S-5 test was used to recover the remaining lethals in this study (see also LINDSLEY and EDINGTON 1957). The chromosomes used in the S-5 method and their essential features are as follows:

(1.) *Ins(1)sc^{8L,81R}*dl-49, *y^{31d} sc^{-} v f B*: This chromosome is doubly inverted and marked with the recessive mutants *y^{31d}*, *v*, and *f* and the dominant marker *B*; in addition, it is deficient for the locus of scute. Deficiency for *sc* is very inviable in the male (STURTEVANT and BEADLE 1936) and is not covered by y^+Y. This chromosome is very similar to Binscy (MULLER 1952) except that it is *sc* deficient and is consequently designated Biny.

(2.) *y l(1)J1^{259} w m f*: *l(1)259* was recovered as a lethal suppressed by y^+Y but not by a normal Y; it has been shown by FRYE (1959) to be allelic to *l(1)J1*.

(3.) *Ins(1)sc^{4L,8R}, S, y sc^{4+8} B w^{a}*: This chromosome is of essentially the same sequential and marker composition as M-5, but it differs from M-5 in that the left end of *In(1)sc^{8}* has been replaced by that of *In(1)sc^{4}* (i.e., it is *y sc^{4}* M-5 designated S-5). This exchange of tips has resulted in the deletion of the majority of the proximal heterochromatin of the X (most of hA, all of hB, NO, hC, and the proximal one third of hD; COOPER 1959) including *bb^{+}*, and the replacement of y^+ with *y*. Owing to its heterochromatic deficiency, this chromosome fails to pair with the Y chromosome regularly during spermatogenesis, and consequently primary nondisjunction is frequent. From a cross of S-5/y^+Y males to *y* females the number of progeny resulting from fertilization by X-, Y-, 0-, and X/Y-bearing sperm were 2509, 1670, 631, and 98, respectively. Thus, the four sperm classes are produced in approximately the following proportions: X:Y:0:X/Y = 51:34:13:2. SANDLER and BRAVER (1954) attributed the inequality in recovery of reciprocal types to meiotic loss of unpaired chromosomes.

The crossing scheme of the S-5 test is presented in Figure 1. Yellow vermilion males are irradiated and crossed to Biny/*y l(1)J1^{259} w m f* females (these females are obtained from a balanced stock containing Biny/*y l(1)J1^{259} w m f* females and *y l(1)J1^{259} w m f*/y^+Y males). The sons of this cross are inviable owing to the failure of a normal Y to cover *sc^{-}* or *l(1)J1^{259}*; consequently, only the *y v*/Biny and *y v*/*y l(1)J1^{259} w m f* daughters survive, and they will be virgin if the parents have been removed. F_1 *y v*/Biny females are selected and crossed individually to S-5/y^+Y males. The progeny of this cross are indicated in the square in Figure 1. The only regular sons expected from this cross carry the original irradiated X chromosome; roughly 75 percent (34/47) of them carry the y^+Y and are consequently y^+ *v*, whereas 25 percent carry no Y and are *y v*. Cultures are scored for the presence of *v* (X/Y) and *y v* (X/0) males. The presence of both classes (Table 1, line 1) indicates a nonlethal culture; the absence of both classes of males (Table 1, line 2) indicates an orthodox lethal; the presence of *v* males but not *y v* males (Table 1, line 3) indicates a change that is viable as an X/Y male but inviable as an X/0 male. The reciprocal of this last class is possible, i.e., where X/0 survives and X/Y is lethal (Table 1, line 4). A few tentative cases have been encountered, but this class of lethal will not be



			PATERNAL GAMETES			
			REGULAR		EXCEPTIONAL	
			S-5 = 0.51	y^+Y = 0.34	0 = 0.13	S-5/y^+Y = 0.02
MATERNAL GAMETES	REGULAR	y v	y v/S-5 y B/+ ♀	y v/y^+Y v ♂	y v/0 y v ♂	y v/S-5/y^+Y B/+ ♀
		Biny	Biny/S-5 y^{31d} sc B/B ♀	Biny/y^+Y dies	Biny/0 dies	Biny/S-5/y^+Y sc B/B ♀
	EXCEPTIONAL	0	S-5/0 dies	y^+Y/0 dies	0/0 dies	S-5/y^+Y sc w^a B ♂
		y v/Biny	y v/Biny/S-5 dies	y v/Biny/y^+Y v B/B ♀	y v/Biny y^{31d} v B/B ♀	y v/Biny/S-5/y^+Y dies

FIGURE 1.—Crossing scheme of the S-5 method for detecting sex-linked recessive lethals whose expression is affected by the Y chromosome and the genotypes and phenotypes expected in the F_2 culture. S-5 = *Ins(1)sc*$^{4L, 81R}$*,S, y sc*^{4} *w*^{a} *B*, and Biny = *Ins(1)sc*$^{8L, 81R}$, dl-49, y^{31d} *sc*$^-$ *v f B*. Orthodox lethal cultures lack both *v* and *y v* males; lethals that are suppressed by the Y produce *v* but not *y v* males; and nonlethal cultures produce both types of males. Exceptional ova are produced only by certain induced rearrangements.

considered further in this communication. Several additional features of this method should be pointed out: (1) Since Biny males fail to survive, nonvirginity is not a problem encountered in retesting lethal cultures. The only putative lethal-bearing cultures in which *y v*/S-5 females might be nonvirgin are where X/Y males survive and X/0 males die. In these cases, the original diagnosis may be confirmed by outcrossing the y^+ *v* sons to XX/0 virgins (where XX designates any compound X chromosome, see NOVITSKI 1954) and the stock may be maintained by crossing the *y v*/S-5 and *y v*/y^+ Y progeny. (2) Since the S-5 chromosome is deficient for the majority of the proximal heterochromatin, any lethal in the proximal heterochromatin should fail to survive in combination with S-5

TABLE 1

Survival of the diagnostic classes of progeny characteristic of the various types of lethals that can be distinguished in the F_2 of the S-5 test, and the ability of the X/S-5/Y females from the S-5 F_2 to produce X*/Y/Y sons*

Lethal type	Diagnostic classes among F_2 of S-5 test			Son of F_2 X*/S-5/Y♀ from S-5 test
	$y\,v\,♂ = X^*/0$	$v\,♂ = X^*/Y$	$y\,B♀ = X^*/S\text{-}5$	X*/Y/Y
Nonlethal	+	+	+	+
Orthodox; not in proximal heterochromatin	—	—	+	—
Suppressed by one Y; not in proximal heterochromatin	—	+	+	+
Y-enhanced	+	—	+	+
Suppressed by one Y; in proximal heterochromatin	—	+	—	+
Orthodox; in proximal heterochromatin	—	—	—	+
Suppressed by two, but not by one, Y	—	—	+	+

(Table 1, line 5). Thus cultures carrying lethals covered by the y^+Y can be checked for the survival of $y\,B$ females, and it can thus be determined whether they carry a proximal heterochromatic lethal. Furthermore, it is possible that deficiencies are produced that include *bb* as well as regions not homologous with the Y; these would behave as orthodox lethals but would fail to survive in combination with S-5 (Table 1, line 6). (3) It is known that lethals may be produced that survive in the presence of two but not of one Y chromosome in the male. The S-5/$y\,v/y^+$Y females in orthodox lethal cultures can be crossed to normal males, and survival of their $y\,v/y^+$Y/Y males scored (Table 1, line 7). This test was not systematically carried out in the experiments reported in this paper, but one such lethal was incidentally recovered. (4) Some lethals are associated with rearrangements characterized by high nondisjunction in the $y\,v$/Biny females. The exceptional females are $y\,v$/Biny/y^+Y or $y\,v$/Biny, and since Biny carries v, they are $v\,B$ or $y^{31d}\,v\,B$, respectively, and can thereby be recognized. Irradiation of y rather than $y\,v$ males in the experiments reported here made the detection of nondisjunction considerably more difficult.

In the initial design we intended to balance the Y-suppressed lethals by crossing $y\,v\,l/y^+$Y males to XX/y^+Y females. It was discovered however that a large fraction of the Y-suppressed lethal X's were male sterile, and they had to be carried balanced to S-5 (or M-5 in the initial experiments). On the basis of absence or presence of the $y\,B$ females, the Y-suppressed lethals may be divided into those affecting chromocentral heterochromatic loci and those not; they may also be classified on the basis of fertility of the y^+ males. So far, all proximal heterochromatic lethals (henceforth referred to as bb^- lethals) have proved to be male fertile; consequently, the Y-suppressed lethals fall initially into three classes (bb^-, bb^+ male fertile, and bb^+ male sterile), and these classes will be treated separately in the following pages.

Recovery and analysis of Y-suppressed lethals

In Table 2 the lethals recovered from a series of exposures of *y* males are broken down into the various classifications outlined in the preceding section; several points of interest emerge from this table. The first is that approximately 20 percent of the lethals recovered are suppressed by the Y chromosome, at least in the 3- and 4-kr experiments. There is then an appreciable fraction of sex-linked recessive lethals that are not recognized by the Muller-5 method. A second point of interest is the small proportion of the Y-suppressed lethals that are in the proximal heterochromatin of the X chromosome (6/52), and finally, although many Y-suppressed lethals are found, the majority of them are male sterile (35/52).

Tests for bb$^+$: Those lethals that fail to survive in combination with S-5 are considered to be located between the right break points of *In(1)sc^4* and *In(1)sc^{8l}*. Although the only locus in this region that is known to be subject to lethal mutation is *bb*, we considered it likely that there are other such loci, including the nucleolus organizer, which BEERMAN (1960) has shown to be necessary for development in Chironomus. Consequently, the phenotype of each lethal that was in the proximal heterochromatin, according to the criterion that it was inviable in combination with S-5, was tested in combination with *bb*. For these tests two different alleles of *bb* were used; both were carried in homozygous stocks, but in one case *bb*/S-5 females were *bb* in phenotype, and in the other case they were lethal. This has been interpreted to indicate that the second allele, which fails to survive in combination with a known deficiency for the locus and which will be designated *bb′*, can differentiate between mutation and deletion of the *bb* locus whereas the first cannot. None of the six proximal heterochromatic lethals were *bb*$^+$; furthermore, each succumbed in combination with *bb′*, but the four tested in combination with *bb* were bobbed. Thus, the chromosomes tested provide no evidence of loci, other than *bb*, in the proximal heterochromatin that are capable of lethal mutation. Furthermore, all the *bb* lethals behave as deficiencies with respect to their survival in combination with *bb′*. Additional evidence that these lethals are associated with heterochromatic deficiencies is that, during

TABLE 2

Recovery and classification of Y-suppressed sex-linked recessive lethals

Dose (kr)	Chromosomes tested	Nonlethal	Lethal			
			Orthodox	Y-suppressed		
				bb$^-$	*bb*$^+$	
					Male fertile	Male sterile
1	323	318	5	0	0	0
2	416	387	26	0	1	2
3	255	235	18	0	0	2
3*	1,624	1,458	129	3	9	24
4	408	354	43	3	1	7

* Lethals recovered from $y^+/y^-\ ac^-$ M-5/y^+Y × XY/0 rather than from y^+/M-5 × S-5/y^+Y.

spermatogenesis, they exhibit varying degrees of primary nondisjunction from the Y chromosome.

Measurement of relative viability: In order to give some indication of the viability of the lethal-bearing chromosomes as X/Y and X/0 males, each was made heterozygous to a balancer chromosome (y^s M-5 in the case of the *bb*⁻ lethals and S-5 in the remainder), and the resulting females were crossed to XY/Y and to XY/0 males; the XY used was $Y^sX{\cdot}Y^L$, *In(1)EN, KS B f v y·KL* y^+ where *KS* and *KL* refer to the fertility complexes of Y^s and Y^L, respectively (terminology of LINDSLEY and NOVITSKI 1959; BROSSEAU 1960). The results for the *bb*⁻ lethals are presented in Table 3. The data in this table require several comments that are also applicable to the two subsequent tables. Ideally, there should be a regular class of progeny that is constant from one cross to the next with which the recovery of lethal bearing males could be compared. In Table 3 the logical reference genotype is S-5/XY but, owing to an ambiguity in the classification of *B*/+ and *B*/*B* in some of the crosses, the frequency of this class could not be counted reliably. Consequently, both classes of regular daughters were combined and used as the reference genotype. In a cross where the maternal normal sequence X does not carry a lethal, the number of sons carrying this X should be about half the number of regular daughters, and the viability ratio (*y* males/regular females) should approximate 0.5. Any recessive inviability of the normal X would reduce the numerator of the viability ratio and, thus, reduce the ratio below 0.5. Dominant inviability, on the other hand, would reduce the denominator as well as the numerator and consequently cause relatively less decrease in the viability ratio than recessive mortality. This uncertainty would not have been encountered had the Bar classification been unambiguous. The difference in recovery of *y* w^a *B* males in the XY/Y and XY/0 crosses is attributable to the well-known reduced viability of *In(1)sc*^{s}/0 males. It is evident from the data presented in Table 2 that the inviability of the *bb*⁻ lethals as X/0 males is absolute, whereas X/Y males seem to show normal viability.

TABLE 3

Viability as X/Y and X/0 males of bb⁻ *lethals from* y l/*M-5 females* × *XY/0 and XY/Y males*

Parental constitution		Progeny						Viability ratio
		Regular				Exceptional		$\frac{y♂♂}{B/+♀+B/B♀}$
Maternal	Paternal	*y*♂	*y* w^a *B*♂	*B*/+♀	*B*/*B*♀	*v f B*♂	*y B*/+♀	
S-5/*l(1)3a*	XY/0	0	91	273	253	0	0	0
	XY/Y	165	133	155	189	1	1	0.48
S-5/*l(1)74*	XY/0	0	40	543	550	0	0	0
	XY/Y	536	436	474	509	0	0	0.55
S-5/*l(1)158*	XY/0	0	57	491	495	0	1	0
	XY/Y	387	296	362	391	0	0	0.52
S-5/*l(1)452*	XY/0	0	45	555	560	0	0	0
	XY/Y	358	299	344	323	0	0	0.54
S-5/*l(1)481*	XY/0	0	32	390	402	1	0	0
	XY/Y	305	237	338	359	2	1	0.44

TABLE 4

Viability as X/Y and X/0 males of bb⁺ *male-fertile, Y-suppressed lethals from* y l/S-5 *females* × *XY/0 and XY/Y males*

Parental constitution		Progeny						Viability ratio
		Regular				Exceptional		
Maternal	Paternal	$y\,♂$	$y\,w^a\,B\,♂$	$B/+♀$	$B/B♀$	$v\,f\,B\,♂$	$y\,B/+♀$	$\frac{y\,♂}{B/+♀ + B/B♀}$
S-5/+	XY/0	741	0	757	715	6	0	0.50
	XY/Y	550	506	598	588	4	0	0.46
S-5/*l(1)11*	XY/0	9	0	148	309	22*	0	0.02
	XY/Y	189	191	180	349	26*	0	0.36
S-5/*l(1)47*	XY/0	0	0	166	292	1	2	0
	XY/Y	101	279	194	339	1	3	0.19
S-5/*l(1)59*	XY/0	0	0	299	299	0	1	0
	XY/Y	167	230	279	295	1	1	0.29
S-5/*l(1)132*	XY/0	2	0	191	220	2	1	<0.01
	XY/Y	137	211	170	188	0	2	0.38
S-5/*l(1)146*	XY/0	0	0	191	218	1	0	0
	XY/Y	66	153	141	214	0	0	0.19
S-5/*l(1)227*	XY/0	0	0	375	332	3	0	0
	XY/Y	127	245	293	292	0	0	0.22
S-5/*l(1)231*	XY/0	1	0	243	246	1	1	<0.01
	XY/Y	99	97	122	103	0	0	0.44
S-5/*l(1)306*	XY/0	0	0	217	193	2	1	0
	XY/Y	158	174	195	239	0	0	0.36
S-5/*l(1)459*	XY/0	0	0	69	78	3	3	0
	XY/Y	2	39	56	86	1	0	0.01

* These males were f^+, and showed an obviously aneuploid phenotype including nicked wings.

Similar data for the bb^+ lethals are presented in Tables 4 and 5; however, in these cases S-5 was used in place of M-5. Consequently, the $y\,w^a\,B$ class is completely absent in the crosses to XY/0 males owing to the inviability of S-5/0. Several points about these data are noteworthy. The bb^+ male-fertile lethals tested uniformly exhibit complete or nearly complete inviability as X/0 males, but as X/Y males their viability ratio ranges from 0.19 in the case of 47 and 146 to 0.44 in the case of 231. The value of 0.01 for 459 is probably in error for some reason as a value of 0.36 was obtained from a $y\,l(1)459/y^z$ M-5 × XY/Y cross. These results indicate that many of the X/0 lethals have reduced viability as X/Y males. The bb^+ male-sterile lethals also show a range of X/Y male viability, with viability ratios ranging from 0.07 for 150 and 216 to 0.51 for 219. This sample of lethals also shows a range of X/0 viability, with ratios ranging from 0 in many cases to as high as 0.13 in the case of 129. This apparent difference in X/0 viability between the male-fertile and the male-sterile lethals is not real. It is simply an apparent result of using male sterility as an additional criterion on which to base selection; it resulted in saving lethals that produced some X/0 males that would have been discarded in instances where the X/Y males were fertile. It seems quite clear that we are dealing with a continuous two-dimensional distribution of the viabilities of X/0 and X/Y males and that our sampling pro-

TABLE 5

Viability as X/Y and X/0 males of bb^s *male-sterile, Y-suppressed lethals from* y l/*S-5 females* × *XY/0 and XY/Y males*

Parental constitution		Number of progeny						Viability ratio	Proportion primary nondisjunction
		Regular				Exceptional			
Maternal	Paternal	$y♂$	$y\,w^a\,B♂$	$B/+♀$	$B/B♀$	$v\,f\,B♂$	$y\,B/+♀$	$\frac{y♂}{B/+♀+B/B♀}$	$\frac{v\,f\,B♂+y\,B/+♀}{v\,f\,B♂+y\,B/+♀+B/+♀+B/B♀}$
S-5/*l(1)3*	XY/0	0	0	85	87	38	19	0	0.25
	XY/Y	67	100	78	117	63	46	0.34	0.36
S-5/*l(1)25*	XY/0	0	0	54	58	35	23	0	0.34
	XY/Y	26	0	23	30	25	24	0.49	0.48
S-5/*l(1)75*	XY/0	1	0	172	244	73	100	<0.01	0.29
	XY/Y	33	104	120	156	48	71	0.12	0.30
S-5/*l(1)129*	XY/0	26	0	103	99	68	65	0.13	0.40
	XY/Y	120	141	137	150	81	78	0.42	0.36
S-5/*l(1)150*	XY/0	0	0	159	159	88	59	0	0.32
	XY/Y	20	120	157	149	120	82	0.07	0.40
S-5/*l(1)163*	XY/0	1	0	154	111	87	61	<0.01	0.36
	XY/Y	10	76	59	90	28	37	0.08	0.30
S-5/*l(1)216*	XY/0	0	0	42	108	54	1	0	0.42*
	XY/Y	6	48	22	62	38	0	0.07	0.47*
S-5/*l(1)219*	XY/0	0	0	268	298	156	124	0	0.33
	XY/Y	244	208	240	235	90	83	0.51	0.27
S-5/*l(1)223*	XY/0	0	0	33	43	0	0	0	
	XY/Y	43	126	95	133	2	3	0.19	0.02
S-5/*l(1)252*	XY/0	2	0	93	104	58	45	0.01	0.34
	XY/Y	121	138	81	182	60	36	0.46	0.28
S-5/*l(1)361*	XY/0	3	0	142	159	124	88	0.01	0.41
	XY/Y	96	90	90	109	76	71	0.48	0.42
S-5/*l(1)451*	XY/0	6	0	176	200	0	3	0.02	<0.01
	XY/Y	71	113	125	121	1	0	0.29	<0.01
S-5/*l(1)453*	XY/0	0	0	122	125	62	82	0	0.37
	XY/Y	61	50	55	82	32	44	0.45	0.36
S-5/*l(1)454*	XY/0	0	0	44	54	20	12	0	0.25
	XY/Y	14	26	28	34	12	5	0.23	0.22
S-5/*l(1)463*	XY/0	15	0	75	117	48	60	0.09	0.36
	XY/Y	17	45	32	42	19	16	0.23	0.32

* Owing to apparent inviability of exceptional females, $2(v\,f\,B♂)$ was used in place of $v\,f\,B♂+y\,B/+♀$ in estimating primary nondisjunction.

cedures have selected for low X/0 viability and relatively higher X/Y viability. This is illustrated by Figure 2, where the correlation between the relative viability of X/0 males (viability ratio of $l/0 \div$ viability ratio of $+/0$) is plotted against the relative viability of XY males (= viability ratio of $l/Y \div$ viability ratio of $+/Y$). Reductions in viability whose expression is unaffected by the Y should be represented by points scattered along the diagonal with slope 1; the sample selected for these studies can be seen to have a slope much below 1, but it seems reasonable to expect that, were the appropriate viability measurements made on a large random sample of irradiated X chromosomes, the range of slopes from 0 to 1 and probably beyond would be represented.

Among the bb^+ lethals it is possible to produce homozygous females in those cases that are male fertile. It has been incidentally recorded in six cases (11, 47, 59, 227, 231, and 306) that homozygous females survive in the absence of a Y chromosome; in two others (132 and 146) homozygous females survive only in the presence of a Y, and in one (459) homozygous females have not been observed.

A second point of interest regarding Tables 3–5 is the incidence of exceptional progeny apparently arising from maternal nondisjunction. Since the only Y chromosome in recent maternal ancestry is y^+Y and its presence in the cross would have been detected, secondary nondisjunction is clearly not involved.

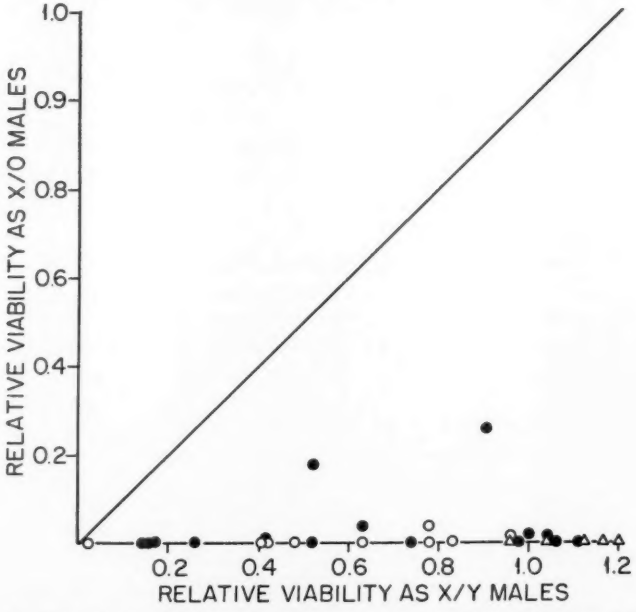


FIGURE 2.—The relative viability as X/0 males versus the relative viability as X/Y males of the lethals tabulated in Table 5. △, bb^- lethals; ○, bb^+ lethals; open symbols represent male-fertile and closed symbols male-sterile lethals.

When an extra Y is present, the lethal X and S-5 proceed to the poles at random. Nondisjunction of the X's in the primary oocyte results in an X/X gamete or a nullo X gamete; the former yields a scorable offspring when fertilized by a nullo X sperm and the latter when fertilized by an X-bearing sperm; consequently, only half the exceptional ova give rise to viable adults. Since the regular daughters constitute about half the products of regular disjunction, primary nondisjunction has been estimated by exceptions/(regular females + exceptions). Nondisjunction of the *bb*⁻ lethal chromosomes from y^2 M-5 is extremely low, and with the possible exception of *l(1)459* the *bb*⁺ male-fertile lethal chromosomes disjoin regularly from S-5. An interesting observation from Table 4 is that, whereas the control crosses produced ten exceptional males and no exceptional females [in accord with the observations of MORGAN, BRIDGES, and STURTEVANT (1925) that exceptional sons resulting from maternal nondisjunction outnumber exceptional daughters], the lethals produced a total of 16 exceptional males and 15 exceptional females. The reason for this is not clear. With two exceptions (223 and 451), the *bb*⁺ male-sterile lethals exhibit an extremely high incidence of nondisjunction, averaging between 30 and 40 percent. The absence of y^2 w^a *B* sons from the cross of *y l(1)25*/S-5 × XY/Y is difficult to explain, but since these data are from a single pair, there is some doubt that this would be repeated with another S-5 chromosome. The absence of exceptional females among the progeny of *y l(1)216* /S-5 females was repeatedly observed and is difficult to account for.

Tests for linkage between the X chromosomes and the autosomes: It was likely that some of the Y-suppressed lethals were rearrangements, and so each was tested genetically for the presence of an X-autosome translocation. Because of the sterility of males carrying the majority of the chromosomes to be tested, it was necessary to carry out the tests in females carrying a dominantly marked and multiply inverted X, 2, and 3. *y l*/S-5/*y*⁺Y females were crossed to *Ins (2L+2R) Cy/In(2LR)Pm, al*^{4} *ds*^{33k} *lt*⁻ *bw*^{V1}; *In(3LR)DcxF/Sb (=Cy/Pm; D/Sb)* males and secondary exceptional *y B Cy*, *y B D*, and *y B Cy D* daughters were recovered and outcrossed, usually to *y w* males. The progeny were counted and the data examined for evidence of new linkage relations. The results of these crosses are summarized in column 6 of Table 6. Approximately half the chromosomes were tested for linkage with chromosome 4 (the *bb*⁻ lethals except 481; 11, 139, and 459 of the *bb*⁺ male-fertile lethals; and the *bb*⁺ male-sterile lethals except 3, 25, 75, 135, 184, 223, and 455); where linkage was encountered, the information is included in column 6 of Table 6. The striking feature of these data is the high incidence of X-autosome translocations among the male-sterile lethals (17/18) and the correspondingly low incidence among the male-fertile lethals (3/16).

Cytological analysis: An analysis of the salivary gland chromosomes of each *bb*⁺ lethal was undertaken, and with one exception, every lethal examined was found to carry a rearrangement involving the X chromosome. The nature of the rearrangement found, and its break points, are summarized in columns 7–9 of Table 6. The salivary configuration of lethals 25, 75, 361, and 463 appears normal; and we inferred, from linkage tests in which these lethals behave as X-autosome translocations, that both break points are in the chromocentral hetero-

TABLE 6

Summary of the cytological and genetic data from the chromosomes studied in this paper

Class	Number*	Relative viability of X/0	Relative viability of X/Y	Nondisjunction in $y\,l$/S-5♀	X chromosome linked to	Cytological data: Type of rearrangement involving X†	Cytological data: Break in X	Cytological data: Break in Autosome
bb⁻	3	0	1.04	<0.01	.	n.t.	.....	..
	74	0	1.20	<0.01	.	n.t.	.....	..
	158	0	1.13	<0.01	.	n.t.	.....	..
	452	0	1.17	<0.01	.	n.t.	.....	..
	456	n.t.†	n.t.	<0.01	.	n.t.	.....	..
	481	0	0.96	<0.01	.	In	12E/F–14B	..
bb⁺ male-fertile	11	0.04	0.78	<0.01	4	Tr	15	101
	47	0	0.41	<0.01	2	Ti	8F–9B	?
	59	0	0.63	<0.01	.	In	3/4–19/20	..
	132	<0.01	0.83	<0.01	.	In	4/5–19/20	..
	139	0	0	<0.01	.	Inp	3C-XR	..
	146	0	0.41	<0.01	.	In	5/6–19/20	..
	227	0	0.48	<0.01	.	In	1/2–19/20	..
	231	<0.01	0.96	<0.01	.	In	1C/D–19/20	..
	306	0	0.78	<0.01	.	Ti	1B/E	?
	459	0	0.02	0.02	2;3	Ring	3D/F–XR	50
bb⁺ male-sterile	3	0	0.74	0.31	3	Tr	4A	81
	25	0	1.06	0.39	2	Tr	19/20	40/41
	75	<0.01	0.26	0.30	2	Tr	19/20	41
	129	0.26	0.91	0.37	2	Tr	18B	41
	150	0	0.15	0.36	2	Tr	16/17	40
	163	<0.01	0.17	0.34	3	Tr	17A/B	80/81
	216	0	0.15	0.44	2;3	n.t.	.....	..
	219	0	1.11	0.30	2	Tr	10A	40
	223	0	0.41	0.02	2	Ti	14F	41–50E
	252	0.02	1.00	0.30	3	n.t.	.....	..
	361	0.02	1.04	0.42	3	Tr	19/20	80/81
	451	0.04	0.63	<0.01	..	.....	.....	..
	453	0	0.98	0.37	3	Tr	12D	80/81
	454	0	0.50	0.23	2;3;4	Tr	12B	80/81
	455	n.t.	n.t.	n.t.	n.t.	Tr	3C	81
	463	0.18	0.50	0.35	3	Tr	19/20	80/81
Other male-sterile	65	1.18	0.85	0.38	3	Tr	16/17	79D
	135	0	0	0.41	2;3	Tr	18/19	41
	184	0	0.01	0.40	n.t.	Tr	18A	81
	220	0.25	0.30	0.41	2;3	Tr	14A	50A;75C

* All chromosomes with numbers below 250 were recovered from $y^{-}\ ac^{-}$ M-5/y/y^{+}Y daughters of irradiated y males and those with numbers above 250 from M-5/y daughters of irradiated y males.

† Not tested.

Tr = reciprocal translocation.
Ti = insertional translocation.
In = paracentric inversion.
Inp = pericentric inversion.

chromatin. Every lethal-associated rearrangement in which all breaks have been localized has at least one break point in the proximal heterochromatin; and, in reciprocal translocations between the X and chromosome 2 or 3, the autosomal break point is chromocentral, whereas the X chromosome break point may be either euchromatic or heterochromatic. This non random distribution of break points is illustrated in Figure 3.

Several of the lethals require additional comment, especially those associated with rearrangements involving more than two break points; in the more complex cases, only the break points of the rearrangements involving the X chromosome and inferred to be lethal associated are presented in Table 6 and Figure 3. Lethal 481 carries, in addition to a *bb* deficiency, a small inversion from 12E-F to 14B that is unassociated with the lethal effect. This can be shown by the recovery of a recombinant that carries the inversion without the lethal, but not its reciprocal, in an X/0 male. Lethal 139 is characterized by its having the portion of the X to the left of 3C7 associated with the chromocenter. Since the proximal portion of X is not capped with any recognizable element, and the X chromosome behaves as a unit in meiosis, *l(1)139* must be a pericentric inversion with a break between 3C5-6 and 3C7. Lethal 139 requires two Y's to survive, and the X/Y/Y males are characterized by white variegation and a phenotype similar to *rst*^{2}. The combination *l(1)139/y w spl sn* variegates for *w* but not *spl*, which is in accord with the transfer of the loci *w* and *rst*, but not *spl*, into chromocentral heterochromatin.

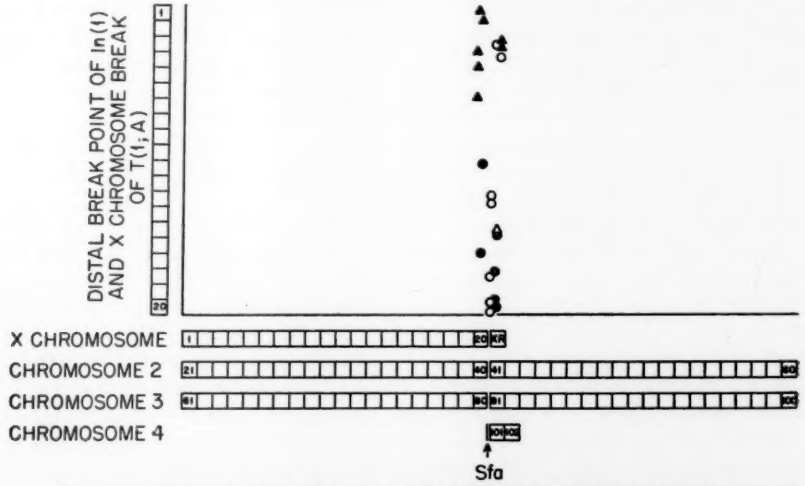

DISTAL BREAK POINT OF In(1) AND X CHROMOSOME BREAK OF T(1;A)
X CHROMOSOME
CHROMOSOME 2
CHROMOSOME 3
CHROMOSOME 4
Sfa
PROXIMAL BREAK POINT OF In(1) AND AUTOSOMAL BREAK POINT OF T(1;A)

FIGURE 3.—The salivary localizations of the break points of the inversion, ring, or translocation associated with each Y-suppressed lethal. Each such rearrangement is characterized by two break points and consequently may be completely represented on Cartesian coordinates. ▲, *In(1)* and X^c; ●, T(1;2); ○, T(1;3); △, T(1;4).

The single recombinant between T(1;4)w^{m5} and *Inp(1)139* loses the roughest and lethal characteristics of *l(1)139*, but retains the mottled phenotype; this crossover replaces the tip of X^R, which is distal to 3C7, with bands 3C4, 5, and 6 as well as most of chromosome 4 from T(1;4)w^{m5}. Lethal 459 is a four-break rearrangement with euchromatic break points at 3D in the X and around 50 in 2R. The proximal part of 2R has been capped by the tip of the X, and both the proximal part of the X and the distal piece of 2R have become attached to the chromocenter. Since, according to linkage tests, chromosome 3 is involved in this rearrangement, it is presumably participating in these chromocentral attachments. The proximal part of the X consistently appears to be a ring chromosome; this is confirmed by neuroblast prophases which reveal a ring as well as five large autosomal elements, three V's, and two rods. It seems that chromosome 3 has been broken into two centric fragments, one of which has captured the tip of 2R and the other of which may have captured X^R. A sperm with a normal complement of arms but with an extra centromere may arise by the splitting of one centromere into two functional fragments (McCLINTOCK 1938) in a normal haploid sperm or by the conversion of a disomic sperm into a haploid sperm with persisting disomy for the centromere region; e.g., in a disomic for 3, one chromosome 3 could be broken immediately to the right and one immediately to the left of the centromere. The acentric fragments could rejoin and, if somewhere else in the complement a ring or a dicentric had been produced, two free acentric fragments would be available for capture by the centric portions of the two chromosomes 3 (i.e., 3L and 3R). It is surprising to note that the preceding lethals represent two cases of breakage in the short arm of the X from a relatively small sample of rearrangements. Lethals 223 and 451 are the only male-sterile lethals not characterized by high primary nondisjunction; they are also the only ones not associated with a reciprocal translocation. Lethal 223 is associated with an insertional translocation of a segment of 2R from 40-50E into 14A of the X; the order of the inserted segment has not been determined. Lethal 451 is not associated with any rearrangement recognizable by either linkage tests or salivary analysis. It does, however, cause an appreciable reduction in recombination in the proximal half of the X; e.g., the *v*—*f* region is reduced from the standard value of 0.237 to 0.120. By recombination measurements the locus of *l(1)451* is inseparable from from th locus of *f*. Lethal 454 is a five-break rearrangement with breaks in 12B, 22–23, 44F, 81, and 101; the new order is tip of X to 12B/81 through centromere of 3 to tip of 3L; tip of 2L to 22–23/12B to centromere of X; tip of 2R to 44F/101 to centromere of 4; and tip of 3R to 81/22–23 through centromere of 2 to 44F/101 to tip of 4.

At the end of Table 6, four translocations are included whose expression is not convincingly affected by the Y chromosome. They are included because (1) in other respects they resemble the bb^+ male-sterile lethals and (2) they demonstrate that (a) most of the properties of this class of lethals are correlated with their translocation nature and (b) Y-suppressed lethality is not a necessary correlate of male sterility or high primary nondisjunction. T(1;3)65 is nonlethal and carries, in addition to the translocation, a large inversion in the X from 1C

to 10B. It is male sterile and associated with high primary nondisjunction. This particular X, when heterozygous to y^2 *cv v f*, produced no recombinants among 725 progeny; it is a very useful balancer for the X. T(1;2;3)135 and T(1;3)184 are orthodox lethals that are characterized by high primary nondisjunction of the X's; there are indications of excessive nondisjunction in about one eighth (8/65) of the orthodox lethals recovered at 3 and 4 kr by the S-5 method. In addition to the T(1;2) indicated in Table 5, *l(1)135* has a reciprocal T(2;3) with break points in 37 and 85A. T(1;2;3)220 is a semilethal characterized by male sterility and high primary nondisjunction; the distribution of the elements in the translocation is X^D2^P, 2^D3^P, and 3^DX^P.

Tests of effect of enhancers of variegation: A number of observations made it seem likely that the *bb*$^+$, Y-suppressed lethals were variegated position effects. To test this suspicion, we measured the viability of each lethal in combination with two different dominant enhancers of variegation, *M(2)S10* (see SCHULTZ in MORGAN, SCHULTZ, and CURRY 1940) and *Evar 7*, that were kindly furnished by DR. JACK SCHULTZ. Females heterozygous for S-5 and the lethal-bearing X were crossed to *In(1)w*^{m4}; *Ins(2L+2R)Cy/Evar 7* or *Ins(2L+2R)Cy/M(2)S10* males and the proportion of *Cy* to non-Cy flies was scored among the lethal- and nonlethal-bearing sons. The data from these crosses are presented in Table 7. Before the lethal-bearing chromosomes were tested, both enhancers were tested against *In(1)w*^{m4} and shown to still be very effective enhancers of the white variegation. Table 7 indicates that recovery of some of the lethals is reduced by *Evar 7* (i.e., 75 and 463), some by *M(2)S10* (i.e., 11, 3, 216, 219, and 453), both enhancers in some cases (i.e., 59, 146, 227, 454, and 455), and neither in still other cases (i.e., 306, 132, 231, 25, 129, 150, 220, 223, 252, and 361). These observations are in accord with those made earlier by SCHULTZ (1950), who found with visible phenotypes that enhancers of variegation may be specific in their action in that particular enhancers are characterized by enhancing a particular array of V-type position effects. The absence of S-5 sons from the *M(2)S10* cross is unexplained as yet, although a plausible explanation is the existence of a Y*bb*$^-$ in the *M(2)S10/Cy* stock. A further indication that we are dealing with position effects in these cases is that, in several cases where the X/Y males have a distinct phenotype, the phenotype was more extreme in the *Evar 7* than in the *Cy* flies.

The morphology of male sterility: Examination of testis preparations of a number of the male-sterile genotypes revealed that the responsible lesion was spermiogenic. Meiosis appeared to be normal; there was evidence of quadrivalent formation, as would be expected from translocation heterozygotes and of frequent nondisjunction, but there were no other meiotic abnormalities noted. The abnormal formation of sperm was first evident at the beginning of sperm head elongation. Whereas normal spermiogenesis produces a progressive elongation and narrowing of the spermatid nucleus with a concomitant loss of cytoplasm resulting in an extremely long, narrow heavily staining structure, condensation of the abnormal structure takes place in the absence of elongation. The mature sperm head is therefore a small, highly chromatic, spherical or ellipsoidal ele-

TABLE 7

Comparison of survival of bb$^+$ *Y-covered lethal-bearing chromosome in the presence and absence of specific dominant enhancers of variegation*

	X/Y sons from *y l*/S-5 ♀ crossed to									
	In(1)w^{m4}/Y; Evar 7/Ins(2L+2R)Cy						*+/Y; M(2)S-10/Ins(2L+2R)Cy*			
	Regular				Exceptional		Regular		Exceptional	
	y	*y Cy*	*y w^a B*	*y w^a B Cy*	+	*Cy*	*y*	*y Cy*	+	*Cy*
l(1)11	43	32	24	42	3	5	14	38	1	22
l(1)59	16	73	98	66	0	0	10	109	1	1
l(1)132	38	32	27	35	0	1	39	50	2	0
l(1)146	28	45	52	47	0	0	23	40	0	1
l(1)227	45	88	118	104	0	1	1	49	5	0
l(1)231	60	63	66	58	1	1	21	33	0	0
l(1)306	32	48	32	48	0	1	8	16	0	0
l(1)459	0	1	8	9	0	0	0	0	1	0
l(1)3	23	34	30	46	20	12	13	29	10	16
l(1)25	37	39	36	46	20	24	8	4	19	25
l(1)75	8	22	32	27	13	19	26	31	31	28
l(1)129	47	42	27	35	20	30	30	38	21	21
l(1)150	5	4	21	23	18	16	6	11	40	57
l(1)163	0	8	51	51	19	25	0	1	29	27
l(1)216	0	0	7	9	1	6	1	13	11	17
l(1)219	58	49	35	51	10	21	12	49	23	44
l(1)220	2	5	8	18	15	20	4	9	17	31
l(1)223	11	30	29	41	2	5	15	27	1	5
l(1)252	19	18	17	20	9	12	6	13	1	7
l(1)361	42	53	65	78	26	37	34	54	27	51
l(1)451	0	0	71	76	5	9	0	0	2	2
l(1)453	30	24	28	28	24	32	14	52	22	36
l(1)454	7	30	24	23	5	11	0	5	5	8
l(1)455	4	14	69	46	45	33	1	4	23	31
l(1)463	3	95	61	66	24	30	30	44	40	28

ment. In many cases, there is evidence of disassociation of the spermatid nucleus from the nebenkern such that, when the sperm were mature, the heads were no longer associated with the tails.

DISCUSSION

It has been shown in the preceding pages that sex-linked recessive lethals whose expression is suppressed by the Y chromosome are a common consequence of radiation. In fact, they constitute approximately 20 percent of all sex-linked recessive lethals produced by 3 and 4 kr. The Y-suppressed lethals were immediately classifiable into two groups on the basis of their ability to survive in combination with *In(1)sc^{4L,8R}*, which is deficient for the majority of Xh [the proximal third of hD, all of hC and hB, and nearly all of hA (see COOPER 1959, Figure 56)]. Irradiated chromosomes that fail to survive in this combination are presumed to carry lethals in the proximal heterochromatin of the X. Their suppression is

attributable to the fact that the Y carries normal loci homologous to the affected loci in the irradiated X. It is of interest to know the proportion of proximal heterochromatic lethals that can be covered by the Y chromosome. Although this question cannot be answered by the data presented in this report, we can surmise that there are loci in Xh for which there are no normal alleles in the Y. An example of such a locus is *su*^{W}-*f* (COOPER 1959). Two lethals were recorded in S-5 experiments that were not covered by the Y and failed to survive in combination with S-5. Presumably, they were deficient for a portion of the X that is homologous with the Y and a portion that is not. It was not determined whether the latter portion was heterochromatic or euchromatic, however. It might be of interest to screen a sample of orthodox sex-linked recessive lethals for survival in combination with a duplication for Xh. It is known that the *In(1)sc*^{4L,8R} deficiency is completely within the region of X-Y homology insofar as lethally mutating loci are concerned, since the deficiency is covered by the Y. There may be heterochromatic loci, however, that are carried by both *In(1)sc*^{4L,8R} and the Y chromosome. Lethal mutation at these loci would produce a mutant that is covered by the Y and would survive in combination with *In(1)sc*^{4L,8R}. Of the lethals studied in this report, six were judged to be in the proximal heterochromatin on the basis of their failure to survive in combination with *In(1)sc*^{4L,8R}. The remaining lethals, which survive in combination with *In(1)sc*^{4L,8R}, differ from these six in many respects and clearly fall into another category; consequently, we have no evidence of any proximal heterochromatic lethal that is covered by the Y and yet survives in combination with *sc*^{4L,8R}. A further point of interest is that all six proximal heterochromatic lethals seem to be deficient for *bb*, judging from their failure to survive in combination with an allele of *bb* that itself dies in combination with a known deficiency for *bb*. Further evidence of their deficient nature is the observation that none of them segregate from the Y chromosome with complete regularity. Thus, our data provide no evidence of loci capable of lethal mutation in the portion of Xh that is homologous to Y, other than *bb*. It seems quite likely that a deficiency for the nucleolus organizer, but not *bb*$^{+}$, would be lethal as BEERMAN (1960) has shown to be the case in Chironomus, but as *bb*$^{+}$ and NO are very closely linked, the chances of separating them are small (COOPER 1959).

The other major class of Y-suppressed lethals is made up of those that survive in combination with *In(1)sc*^{4L,8R}. These lethals are not confined to the proximal portion of the X but are scattered over its entire length. They are further distinguished from the proximal heterochromatic lethals in that, with one exception, all are associated with gross rearrangements involving the X chromosome in which at least one break is in the chromocentral heterochromatin. These observations agree with the hypothesis that these are variegated position-effect lethals (LEWIS 1950). This explanation is strengthened by the observation that the expression of many of these lethals in XY males is enhanced by specific autosomal dominant enhancers of variegation. Finally, in several cases where the test was possible, we found that females homozygous for the lethal-bearing X survive in the absence of a Y chromosome. It is a common observation that the effect of Y chromosomes on the expression of variegation is related, not to the absolute

numbers of Y's present, but to the number of Y's in relation to a normal constitution; that is, it is a departure from the normal heterochromatin-euchromatin balance and should, as COOPER (1956) has postulated, affect the expression of V-type position effect, so that the phenotypic expression may roughly be seriated X/0 > X/Y = X/X > X/Y/Y = X/X/Y. The survival similarity of homozygous females to X/Y rather than X/0 males, then, accords well with phenotypic observations on variegated position effects. These arguments have led us to the conclusion that the second category of lethals are variegated position effects.

The location of the break points of the variegated lethals agrees well with previous experience with V-type position effects. The inversions have the distal break point in the euchromatic portion of the X (Xe) and the proximal break in chromocentral heterochromatin of the X (Xh). The translocations may have their X break in either Xe or Xh, but the autosomal break point is invariably in the chromocentral heterochromatin (Ah). We can probably generalize from these observations that the chromosomal realignments that are sex-linked variegated lethal compatible are Xe–Xh, Xe–Ah, and Xh–Ah, and those that are not are Xe–Xe, Xe–Ae, and Xh–Ae. Variegation for loci in the proximal heterochromatin, if indeed that is the explanation of lethality in the T(Xh;Ah) cases, is not a generally observed result of a wholly heterochromatic rearrangement in *D. melanogaster*. In a total of 193 *R(ci*$^+$*)*'s, KHWOSTOVA (1939) observed translocation of chromosome 4 to Xh distal to *bb*$^+$ in only 12 cases and to the distal regions of the Y in 19 cases, but not to any other chromocentral region. In similar experiments with the light locus, HESSLER (1958) found among 35 *R(lt*$^+$*)* variegated effects that each had one break in region 40 of 2L, but that in no case was the second break in chromocentral heterochromatin. In the present study, however, 4/17 of the postulated V-type position effect translocations have both break points in the chromocentral heterochromatin. These cases presumably provide additional examples of heterochromatic loci whose variegation is suppressed rather than enhanced by the Y chromosome (GRELL 1959).

The present experiments demonstrate that approximately 18 percent of the lethals produced by 3 and 4 kr are V-type position effects that can be made to survive by adding a single Y chromosome to the complement. Experiments currently in progress indicate that about 4 percent of the remainder can be made to survive by adding a second Y. This means that relatively few of the lethals sampled by orthodox procedures (i.e., as X/Y males) can be shown to be V-type position effects by suppression with an extra Y. We might question how closely these procedures have estimated the contribution of position effect lethals to all sex-linked recessive lethals and to inquire into the importance of position effect in semilethal and subvital changes. The observed proportion of about 21 percent position-effect lethals probably underestimates the importance of position effects in induced viability changes. Our methods have tended to select effects that can be converted from essentially complete inviability to some degree of viability by the addition of a Y chromosome and to ignore effects that can be changed from low viability to higher viability by addition of a Y (see, however, lethals 129 and 463). Also remaining undetected were any position effects sufficiently extreme

that the suppression afforded by two Y's was insufficient to impart viability to them. Furthermore, it is clear that stable (S-type) position effects have been entirely overlooked, since their expression is unaffected by Y chromosome constitution. MULLER (1948) has reported evidence for induced S-type position effect lethals, but since he presents no data, we have no way of estimating their contribution.

The fact that 20 percent of the chromosomes lethal as X/0 males survive as X/Y males, whereas only four percent of the lethals that are lethal as X/Y males survive as X/Y/Y males indicates that the first Y has a relatively much greater effect on the expression of variegated lethals than the second Y. This observation is understandable because the addition of a Y chromosome to an X/0 male causes a proportionately greater increase in the heterochromatic complement than the addition of a Y to an X/Y male.

The V-type position-effect lethals are further divisible into two groups on the basis of fertility of X/Y males. We believe that this subdivision is less related to the nature of lethality than to the type of rearrangement associated with lethality. A fairly accurate generalization from the observations is that the inversions and insertional translocations are male fertile, and the reciprocal translocations are male sterile. The association of sterility with homozygosis for reciprocal translocations has been observed since the early thirties (e.g., PATTERSON, STONE, BEDICHEK and SUCHE 1934), but to the best of our knowledge, SCHULTZ (1947) was the first to point out the strong correlation between male sterility and X-chromosome rearrangements, specifically reciprocal translocations between the X and the autosomes. He considered that sterility was the consequence of gene mutation near the point of breakage or, more probably, of position effect. The position effect explanation of this sterility is also invoked by LEWIS (1950). The present observations disagree with the position effect as well as the mutational hypothesis of male sterility. Our sample of variegating lethals includes inversions and reciprocal and insertional translocations, and, though all are V-type position effects with regard to their lethal phenotype, only the reciprocal translocations are associated with male sterility. Furthermore, we found that the addition of a second Y chromosome has no effect on fertility (lethals 361, 453, and 455 tested as X/Y/Y males). One attribute that is shared by the sterilizing rearrangements found in the present experiments is disruption of the continuity between the two ends of the X chromosomes, and it may be that the linear continuity of the X plays some role in normal spermiogenesis. The situation cannot actually be so simple since there is not a perfect correlation between disruption in the continuity of the X chromosome and loss of male fertility; there are four apparent exceptions in the present material. Lethal 11 is a T(X;4) that is male fertile; examination of the known T(X;4)'s listed in BRIDGES and BREHME (1944), however, reveals that none are listed as sterile and, although this may be an artifact of the method of recovering the T(X;4)'s, it is also possible that the centromeres of X and 4 are interchangeable, so that a T(X;4) does not constitute an effective disruption of continuity. This same reservation also seems to apply to T(X;Y)'s (NICOLETTI and LINDSLEY 1960) where the T(X;Y)'s that are T(X;Y)/Y sterile are nearly

all T(X;Y;A)'s, whereas no T(X;Y)/Y fertile case is a T(X;Y;A). Lethal 459 is a quasi-reciprocal translocation that results in a disruption of the continuity of the X chromosome but is male fertile; the portion of the X distal to region 3D is translocated to 2R, but the proximal portion of the X forms a ring rather than capturing the tip of 2R. T(1;2) 223 is an insertional translocation; yet it is male sterile. Since the sterility of this rearrangement is caused by variegation for missing external genitalia rather than abortive spermiogenesis, in a sense it may be considered male fertile. Finally, 451 is male sterile and unassociated with any cytologically detectable rearrangement. This chromosome is a mystery; genetic tests show no evidence of a translocation, but there is some reduction of crossing over in the region of *f* and the lethal seems to be inseparable from f^+. Spermatogenesis appears to be normal, but the mature sperm are not motile.

SCHULTZ (1947) has attempted to establish some generalizations describing the relation between break points of reciprocal T(X;A)'s and male fertility. He claimed that, when both the X and the autosomal break point are heterochromatic, the translocation is usually male fertile; whereas, when both breaks are euchromatic, the translocation is usually male sterile. The situation for heterochromatic-euchromatic translocations is simply described as being more complex. Because the current sample was selected on the basis of variegated lethality, the autosomal break of each reciprocal T(X;A) is in the chromocentral heterochromatin; and furthermore, in one third (4/12) of the cases, the X break also is heterochromatic. The finding that all these cases are male sterile fails to support SCHULTZ's generalizations, but, since selection of translocations has been biased with respect to break points, the present material does not supply information that would be valuable in formulating new generalizations.

High primary nondisjunction of the lethal-bearing X from a balancer X (S-5) is another characteristic in addition to male sterility that is associated with the reciprocal T(X;A)'s studied here. The only male-sterile lethals not characterized by high primary nondisjunction were 223 and 451, and these are the two male steriles that are not associated with a reciprocal T(X;A). Similar high nondisjunction frequency is observed when the S-5 chromosome is replaced with a normal sequence, but the incidence of exceptions is about half that observed with S-5. Since a variable proportion of nontransmissible products of meiosis results from translocation heterozygosis, it is not possible to estimate the incidence of nondisjunction of the X's (i.e., 3–1 disjunction) among all primary oocytes from the incidence of exceptions among the viable progeny. The qualification must be added to the estimates of nondisjunction in Table 4 that primary nondisjunction of the X chromosomes is being estimated for only those cells that produce orthoploid segregations for the autosome involved in the T(X;A). It is possible that selecting T(X;A)'s with chromocentral autosomal break points has tended to minimize the orderly pairing and separation of the X's, although T(1;3)65, which does not have a chromocentral break point, is also characterized by a high incidence of exceptions. DOBZHANSKY (1932) observed a similar high incidence of primary exceptions for the sex chromosomes from attached-X females carrying T(Y;2)'s; and indeed, judging from the break points (WHITTINGHILL 1937), he

did find that a high incidence of exceptions was correlated with a chromocentral break in chromosome 2. Furthermore, CROUSE (1960) has recently reported on a reciprocal translocation between the X chromosome and chromosome 2 in *Sciara coprophila* that produces high primary nondisjunction of the X chromosomes in heterozygous females.

SUMMARY

A method has been devised that allows the detection and recovery of sex-linked recessive lethals whose lethal phenotype is suppressed by the Y chromosome, as well as orthodox lethals that are inviable with or without a Y. With doses of 3 and 4 kr, approximately 20 percent of all induced sex-linked recessive lethals survive in the presence of a Y chromosome and are consequently overlooked by currently used methods of lethal detection. The Y-suppressed lethals may be divided into two major classes. About one eighth of them are deficiencies for the proximal heterochromatin, specifically for bb^+, and they are suppressed by the Y because the Y carries the homologous region. The other seven eighths are nearly all associated with gross rearrangements with at least one break point in the chromocentral heterochromatin and at least one in the X. These are concluded to be variegated position-effect lethals. The V-type position-effect lethals may be further subdivided into one group that is male fertile and associated with X chromosome inversions or insertional translocations and another group that is male sterile and associated with reciprocal translocations with a break in the X and a break in the chromocentral heterochromatin of chromosome 2 or 3. The sterility of the reciprocal translocations is attributable to failure of sperm head elongation. It is postulated that continuity of the X chromosome plays an important role in normal spermiogenesis and that this continuity is disrupted by reciprocal translocation.

LITERATURE CITED

BEERMAN, W., 1960 Der Nukleolus als lebenswichtiger Bestandteil des Zellkernes. Chromosoma **11**: 263–296.

BRIDGES, C. B., and K. S. BREHME, 1944 *The Mutants of Drosophila melanogaster*. Carnegie Inst. Wash. Publ. **552.**

BROSSEAU, G. E., 1960 Genetic analysis of the male fertility factors of the Y chromosome of *Drosophila melanogaster*. Genetics **45**: 257–274.

COOPER, K. W., 1956 Phenotypic effects of Y chromosome hyperploidy in *Drosophila melanogaster*, and their relation to variegation. Genetics **41**: 242–264.

1959 Cytogenetic analysis of the major heterochromatic elements (especially Xh and Y) in *Drosophila melanogaster*, and the theory of "heterochromatin." Chromosoma **10**: 535–588.

CROUSE, H. V., 1960 The nature of the influence of X-translocations on the sex of progeny in *Sciara coprophila*. Chromosoma **11**: 146–166.

DOBZHANSKY, TH., 1932 Studies on chromosome conjugation. I. Translocations involving the second and the Y chromosomes of *Drosophila melanogaster*. Z. Ind. Abst. Vererb. **60**: 235–286.

FRYE, S. H., 1959 Further investigation concerning the frequency and analysis of minute rearrangements of the yellow region in relation to X-ray dose in *Drosophila melanogaster*. (Abstr.) Genetics **44**: 511.

GOWEN, J. W., and E. H. GAY, 1934 Chromosome constitution and behavior in eversporting and mottling in *Drosophila melanogaster*. Genetics **19**: 189–208.

GRELL, R. F., 1959 The Dubinin effect and the Y chromosome. Genetics **44**: 911–922.

HESSLER, A. Y., 1958 V-type position effects at the light locus in *Drosophila melanogaster*. Genetics **43**: 395–403.

KHWOSTOVA, W. W., 1939 The role played by the inert regions of chromosomes in the position effect of the cubitus interruptus gene in *Drosophila melanogaster*. Izvest. Akad. Nauk S.S.S.R. Ser. Biol. **4**: 541–574.

LEWIS, E. B., 1950 The phenomenon of position effect. Advances in Genet. **3**: 73–115.

LINDSLEY, D. L., and C. W. EDINGTON, 1957 A screening method for recovering sex-linked recessive lethals whose expression is influenced by the Y chromosome. Drosophila Inform. Serv. **31**: 131–132.

LINDSLEY, D. L., and E. NOVITSKI, 1959 Compound chromosomes involving the X and Y chromosomes of *Drosophila melanogaster*. Genetics **44**: 187–196.

MCCLINTOCK, B., 1938 The production of homozygous deficient tissues with mutant characteristics by means of the aberrant mitotic behavior of ring-shaped chromosomes. Genetics **23**: 315–376.

MORGAN, T. H., C. B. BRIDGES, and A. H. STURTEVANT, 1925 The genetics of Drosophila. XI. Nondisjunction of the X chromosomes. Bibl. Genet. **2**: 109–129.

MORGAN, T. H., J. SCHULTZ, and V. CURRY, 1940 Investigations on the constitution of the germinal material in relation to heredity. Carnegie Inst. Wash. Ybk. **39**: 251–255.

MULLER, H. J., 1948 The construction of several new types of Y chromosomes. Drosophila Inform. Serv. **22**: 73–74.

1950 Some present problems in the genetic effects of radiation. J. Cellular Comp. Physiol. (Suppl. 1) **35**: 9–70.

1952 Breeding systems for detection of sex-linked lethals in successive generations. Drosophila Inform. Serv. **26**: 113–114.

NICOLETTI, B., and D. L. LINDSLEY, 1960 Translocations between the X and the Y chromosomes in *Drosophila melanogaster*. Genetics **45**: 1705–1722.

NOVITSKI, E., 1954 The compound X chromosomes in Drosophila. Genetics **39**: 127–140.

PATTERSON, J. T., W. STONE, S. BEDICHEK, and M. SUCHE, 1934 The production of translocations in Drosophila. Am. Naturalist **68**: 359–369.

SANDLER, L., and G. BRAVER, 1954 The meiotic loss of unpaired chromosomes in *Drosophila melanogaster*. Genetics **39**: 365–377.

SCHULTZ, J., 1941 The function of heterochromatin. Pp. 257–262. Proc. 7th Intern. Congr. Genet. Edited by R. C. PUNNETT. (Suppl. J. Genet.) Cambridge Press. Cambridge, England.

1950 Interrelations of factors affecting heterochromatin-induced variegation in Drosophila. (Abstr.) Genetics **35**: 134.

1947 The nature of heterochromatin. Cold Spring Harbor Symposia Quant. Biol. **12**: 179–191.

STURTEVANT, A. H., and G. W. BEADLE, 1936 The relation of inversions in the X chromosome of *Drosophila melanogaster* to crossing over and disjunction. Genetics **21**: 554–604.

WHITTINGHILL, M., 1937 Salivary studies on translocations. Drosophila Inform. Serv. **8**: 82–84.

MEIOTIC DRIVE IN NATURAL POPULATIONS OF DROSOPHILA MELANOGASTER. V. ON THE NATURE OF THE SD REGION[1,2]

L. SANDLER AND YUICHIRO HIRAIZUMI

Department of Genetics, University of Wisconsin, Madison, Wisconsin

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SECOND chromosomes were collected from nature which, when heterozygous with a normal chromosome II in males, are present in functional sperm much more often than the expected 50 percent. This phenomenon, named segregation-distortion, was found to depend on a locus named Segregation-distorter (symbolized *SD*), which is located near the centromere (and probably in the right arm) of chromosome II.

Studies on the formal genetics of *SD* have been reported by SANDLER, HIRAIZUMI and SANDLER (1959) and by SANDLER and HIRAIZUMI (1959, 1960). From the evidence presented there, the following propositions, relevant to the present discussion, appear to be true.

I. The phenomenon of segregation-distortion is operative only in heterozygous *SD* males—not in homozygous *SD* males nor in females.

II. The actual distorted ratios may be visualized as resulting from some sort of misreplication (formally equivalent to a chromosome break) of the SD^+-bearing chromosome, conditioned by *SD*, which somehow causes a large proportion of the SD^+-containing spermatocytes to behave abnormally so that functional SD^+-bearing sperm are not produced.

III. In order for *SD* to have this effect, the *SD* region must synapse with the SD^+ region of the homolog. Thus, when *SD* is heterozygous with a structurally rearranged homolog, as for example the Curly inversion, *In(2LR)Cy*, segregation is normal.

IV. At, or near, the tip of IIR there is a stabilizing modifier of *SD*, *St(SD)*. In the presence of this modifier, *SD* is *stable* and the segregation ratio is constant and high (often 20:1 or more) from male to male; in the absence of *St(SD)*, *SD* action becomes somewhat variable *(semistable)* and, rather frequently, changes spontaneously to a highly variable state *(unstable)*.

V. If *SD*, from certain *SD* lines, is passed from a male into a female (so balanced that crossing over in chromosome II does not occur), and *SD*-bearing male progeny are collected from such individual females, two types of male sibships are observed. From some females (called "unconditioned"), the sons produce the expected distorted ratios in their progeny; from others (termed "conditioned"), one half of the sons produce the expected distorted ratios whereas the other half produce a normal 1:1 segregation. In the next generation, however,

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all males distort irrespective of whether or not their father distorted. This phenomenon has been named conditional distortion.

VI. Normal, *SD*$^+$-bearing, second chromosomes often differ one from another in their sensitivity to the distorting action of *SD*. These differences seem localized in the *SD*$^+$ region.

The present study is concerned with the structure of the *SD* region. Data have been collected that support the conclusions that there are at least two separable elements that function in respect of the phenomenon of segregation-distortion. One of these is the *SD* locus itself which is responsible for the misreplication on the *SD*$^+$-bearing homolog. Closely linked and to the right of *SD* there is an Activator of *SD*, *Ac(SD)*, that is necessary in order for *SD* to operate. Both *SD* and *Ac(SD)* are located within a small chromosomal aberration that (a) causes a striking reduction in crossing over in the *SD* region and (b) directs the activated *SD* locus to cause a misreplication in the homolog of the chromosome carrying the aberration.

The experimental evidence supporting this interpretation of the *SD* region is presented here.

Crossing over in the SD *region:* Crossing over between *pr* and *cn* has been measured in heterozygous *SD* and homozygous *SD*$^+$ females. The markers, purple and cinnabar, span both the centromere and the *SD* locus, with *cn* (on the standard map) being five times as far to the right of the centromere as *pr* is to the left. Normal, *SD*$^+$-bearing second chromosomes of various sensitivities were made heterozygous with a chromosome marked by *pr cn* (which, itself, is rather insensitive) in females and crossed to *pr cn* males. In addition, *SD*-bearing chromosomes of three types were made heterozygous with *pr cn* in females and crossing over measured. These three types were: (1) *R(SD)-4*, which arose by recombination from an original *SD* chromosome and which carries no gross rearrangements, carries the mutant allele of *bw*, (brown) and, since it has been inherited from the male parent, would produce the standard semistable distribution of *k* values if tested in males (Proposition IV); (2) the same *R(SD)-4* inherited from female parents, thus resulting in a proportion of cases (i.e. one half of the *SD* offspring of conditioned females) which, if tested in a male, would have shown no distortion (Proposition V); and finally, (3) *R(SD)-4*, *St(SD)*, a standard *R(SD)-4* onto which has been placed the stabilizing modifier, *St(SD)*, resulting in a line which, when inherited through males, produces a standard stable distribution of *k* values (the parameter *k* is defined as the proportion of functional *SD*-bearing sperm produced by a heterozygote) in sons (Proposition IV). The results of these tests are presented in Table 1.

It can be seen that the control frequency of recombination (i.e. in homozygous *SD*$^+$-bearing females) is relatively constant irrespective of the sensitivity of the *SD*$^+$-bearing chromosome. Similarly the crossover values are constant in heterozygous *SD* individuals regardless of the "distorting state" of *SD*. Crossing over in heterozygous *SD* females, however, is uniformly very much lower than in homozygous *SD*$^+$ females. The reduction in recombination thus appears to depend on

TABLE 1

Crossing over between pr *and* cn *in crosses of females heterozygous for* pr cn *and various* SD *and* SD$^+$*-bearing second chromosomes, by* pr cn *males*

Constitution of parental females*	Phenotype of progeny				Percent recombination
	pr cn	+	*pr*	*cn*	
Ty/ pr cn	4022	4424	52	45	1.14
Canton-S/ *pr cn*	1465	1530	17	17	1.12
Canton-S/ *pr cn*	4158	4561	53	44	1.10
Su(SD), bw/ pr cn	3707	3769	52	46	1.29
al/ pr cn	5462	6653	85	61	1.19
Mean					1.17
R(SD)-4/ pr cn	1581	1689	1	0	0.03
R(SD)-4/ pr cn	3831	3964	3	3	0.08
R(SD)-4/ pr cn	4116	4166	6	4	0.12
R(SD)-4, bw$^+$ St(SD)/ pr cn	5353	6552	7	8	0.13
Mean					0.09

* *Ty* (Tokyo) is a very sensitive wild-type strain derived from a collection near Tokyo, Japan. Canton-S is a standard inbred strain of intermediate sensitivity; the two Canton-S experiments differ only in that the two sets were done at different times. *Su(SD), bw* is a second chromosome carrying the recessive allele of *bw* and a very insensitive *SD*$^+$ region (from the *bw^D* strain). The *al*-bearing second chromosome is also rather insensitive. The first two *R(SD)-4* experiments were done at different times, but in both cases the *SD*-bearing chromosome was inherited from the male parent. The third *R(SD)-4* experiment differed in that the *SD*-bearing chromosome was inherited from the female parent (so balanced, however, that no crossing over occurred in those females). All three tests involved the standard, semistable, *R(SD)-4*. In the final test, a *R(SD)-4* chromosome, made stable by the addition of the stabilizing modifier, *St(SD)*, was used.

the presence of the *SD* region and does not vary depending on the *k* value distribution characteristic of the chromosome when tested in males.

Further tests show that this depression in crossing over is almost—if not exclusively—confined to the right arm of chromosome II, and furthermore, the suppression effect rapidly decreases in magnitude with increasing distance of the measured region from *SD*. These two inferences come from the results of matings between females heterozygous for *R(SD)-4* and a chromosome marked by *b pr cn en* and males *b pr cn en*. The marker black is located to the left of *pr* while engrailed is located to the right of *cn*. The control in this case is *Ty/b pr cn en* females by homozygous recessive males. The results are shown in Table 2.

Here it can be seen that the *b pr* region is not significantly different in the experimental and control sets, the *pr–cn* region shows the dramatic reduction in the heterozygous *SD* set as before, and the *cn–en* region shows a reduction in recombination in the experimental set to about one half that of the controls. Thus it is clear that the depression in crossing over is almost certainly a function of the *SD* region itself and that this depression is confined to a localized region in the right arm of chromosome II.

Characterization of recombinants: From crosses of *R(SD)-4/pr cn* females by homozygous *pr cn* males, 25 *pr* recombinants (symbolized *R(pr)* followed by an identifying number) and 25 *cn* recombinants (symbolized *R(cn)* followed by an identifying number) were selected for further study. These recombinants were backcrossed to the standard *cn bw* strain for a number of generations to insure uniformity of genetic background and then tested (1) for their ability to distort

TABLE 2

Crossing over between b *and* pr *(region I),* pr *and* cn *(region II) and* cn *and* en *(region III) in crosses of females heterozygous for* b pr cn en *and* Ty, *in one case, and,* R(SD)-4 *in the other, by* b pr cn en *males*

Phenotype of progeny	Constitution of female parent	
	Ty/ b pr cn en	*R(SD)-4/ b pr cn en*
b pr cn en	3939	2522
+	5050	3256
b	158	93
pr cn en	150	75
b pr	72	4
cn en	75	3
b pr cn	281	73
en	209	61
b cn en	4	1
pr	9	0
b en	9	4
pr cn	8	1
b pr en	1	0
cn	0	0
b cn	0	0
pr en	0	1
Percent crossovers		
Region I	3.39	2.87
Region II	1.62	0.15
Region III	5.10	2.30

the standard, sensitive, *cn bw* second chromosome, and (2) for their sensitivity to *SD*-5—a stable *SD*-bearing chromosome. The results of these tests are given in Table 3, 4, 5, and 6. The recombinants in Table 3 are separated according to their sensitivity, as shown in Table 5. Similar groupings are made in Tables 4 and 6.

It is immediately evident from these results that *SD* is not a simple locus for the reason that, while none of the *pr* recombinants exhibit segregation-distortion, only about one half of the *cn* recombinants distort (Tables 3 and 4). There are two types of *pr* recombinants recovered—those that do not distort and are insensitive and those that also do not distort, but are of intermediate sensitvity. There are, on the other hand, three distinct types of *cn* recombinants—nondistorting, almost completely sensitive; slightly distorting and somewhat less sensitive; and those that distort and exhibit complete insensitivity. This last class while all clearly exhibit segregation-distortion, are nevertheless all "weaker" distorters than the original *R(SD)-4* that typically exhibits a mean k value of about 0.93. It may be that all of these lines have become unstable, since the semistable *R(SD)-4* does, on occasion, become unstable (Proposition IV), but it seems more probable that the *SD* region in these distorting *cn* recombinants has been changed as a consequence of the recombination. We shall refer to the distorting *cn* recombinants as *SD*, to the slightly distorting *cn* lines as SD^{sa} (*SD*-semiactive) and to the nondistorting *cn* recombinants as SD^{in} (*SD*-inactive).

Another line of evidence indicating that the *SD cn* recombinants carry a changed *SD* region is as follows. It has been shown (Proposition I) that an *SD*-bearing chromosome is completely insensitive to the distorting action of another *SD* chromosome. Although the data in Table 6 indicate that the *SD cn* recombinants (as defined in the preceding paragraph) are indeed insensitive, there were in fact a small number of highly sensitive cultures in some *SD cn* recombinant lines. These cultures were omitted from the tabulations in Table 6, but their presence definitely indicates a change from normal *SD* behavior in these *cn* recombinants.

The change in the nature of *SD* apparently requires a crossover in the immediate vicinity of *SD*. This is evidenced by the fact that ten *b* recombinants and ten *cn* recombinants from *b pr cn en/R(SD)-4* females were tested, and all could be shown to carry an unchanged *SD*.

A sample of each type of *pr* recombinant was made heterozygous with a normal

TABLE 3

The results of tests for segregation-distortion by pr *recombinants from* pr cn/R(SD)-4 *females. The testcross is:* R(pr)/ cn bw♂♂ × cn bw♀♀

Recombinant controls*	Phenotype of progeny *cn bw* 1802	+ 2188	*k* 0.55
1	627	769	0.55
2	634	769	0.55
3	614	700	0.53
6	603	730	0.55
11	706	830	0.54
50	659	799	0.55
52	725	886	0.55
54	966	1228	0.56
70	810	946	0.54
72	1028	1294	0.56
75	508	549	0.52
80	564	752	0.57
82	426	545	0.56
Mean			0.55
4	643	762	0.54
5	684	759	0.53
9	630	646	0.51
10	701	860	0.55
51	822	984	0.54
61	1174	1371	0.54
64	648	728	0.53
71	762	1048	0.58
73	1215	1415	0.54
74	645	671	0.51
81	220	175	0.44
83	293	363	0.55
Mean			0.53

* *cn bw*/ +♂♂ × *cn bw* ♀♀.

chromosome marked by *cn* in females, and crossing over between *pr* and *cn* was measured. Similar tests were made of females heterozygous for each type of *cn* recombinant and a normal chromosome marked by *pr*. The results of these tests are presented in Table 7. From these data, the following inferences can be drawn. (1) The element contributing the major component to the crossover suppression in the *SD* region is associated with the insensitivity of *SD*, because the insensitive *pr* recombinants and the insensitive *cn* recombinants (those that are *SD*) show a level of crossing over comparable to the original heterozygous *SD* lines, while crossing over in the more sensitive *pr* and *cn* recombinants is uniformly much higher. (2) It is not possible to separate SD^{in} from SD^{sa}, among the *cn* recombinants, on the basis of crossing over. (3) All of the recombinants have been changed by the crossover in the vicinity of *SD*, as is evidenced by the fact that crossing over is lower in all of the tested recombinants than in the controls. Thus all of the

TABLE 4

The results from tests for segregation-distortion by cn *recombinants from* pr cn/ R(SD)-4 females. *The testcross is:* R(cn)/ cn bw ♂ ♂ × cn bw ♀ ♀

Recombinant controls*	Phenotype of progeny *cn bw* 1858	*cn* 2264	*k* 0.55
3	369	1810	0.83
8	373	1075	0.74
9	183	2105	0.92
15	321	1654	0.84
16	552	1459	0.73
19	433	2025	0.82
53	172	1209	0.88
57	305	2118	0.87
59	389	1585	0.80
62	589	1757	0.75
65	321	1568	0.83
Mean			0.82
1	744	1031	0.58
5	1571	2964	0.65
6	979	1294	0.57
12	1191	1633	0.58
55	631	910	0.59
Mean			0.59
2	822	937	0.53
4	1177	1268	0.52
7	1177	1278	0.52
10	1190	1356	0.53
13	826	893	0.52
14	1218	1327	0.52
17	1013	1192	0.54
18	1229	1484	0.55
20	1083	1258	0.54
Mean			0.53

* *cn/cn bw* ♂♂ × *cn bw* ♀♀.

TABLE 5

Tests for the sensitivity of pr *recombinants from* pr cn/ R(SD)-4 *females. The testcross is:* SD-5/R(pr) ♂♂ × pr cn♀♀

Recombinant controls*	Phenotype of progeny *pr* 1477	+ 1833	*k* 0.55
1	656	855	0.57
2	496	569	0.53
3	667	695	0.51
6	568	742	0.57
11	486	835	0.63
50	437	424	0.49
52	343	579	0.63
54	501	611	0.55
70	536	692	0.56
72	689	826	0.55
75	681	799	0.54
80	771	930	0.55
82	380	441	0.54
Mean			0.56
4	260	1174	0.82
5	383	940	0.71
9	347	836	0.71
10	124	1005	0.89
51	301	1399	0.82
61	167	1195	0.88
64	300	803	0.73
71	756	2047	0.73
73	342	756	0.69
74	291	558	0.66
81	443	893	0.67
83	501	1348	0.73
Mean			0.75

* *pr*/+ ♂♂ × *pr cn* ♀♀.

recombination observed that has occurred between *pr* and *cn*, in heterozygotes for *SD*, occurs in the crossover suppressing element of the *SD* region.

A summary of the number and types of recovered recombinants is given in Table 8.

On the nature of the crossover reduction: If the crossover reduction in heterozygous *SD* females is due to a small chromosomal aberration in the vicinity of *SD*, then this crossover reduction should be a reflection of heterozygosity for the aberration. Accordingly, two experiments to test this point were performed. First, various *pr* recombinant females were made heterozygous with a number of *cn* recombinants, and crossing over between *pr* and *cn* was measured. These results are presented in Table 9. It can be seen that heterozygotes for *pr* and *cn* recombinants, each of which showed fairly high crossing over when heterozygous with a normal chromosome, exhibit a frequency of crossing over also in the intermediate range, but lower than either shows when heterozygous with a normal chromo-

TABLE 6

Test for the sensitivity of cn *recombinants from* pr cn/ R(SD)-4 *females. The testcross is:* SD-5/ R(cn) ♂♂ × pr cn ♀♀

Recombinant control*	Phenotype of progeny		k
	cn	+	
	1256	1366	0.52
3	366	357	0.49
8	223	262	0.54
9	301	338	0.53
15	512	497	0.49
16	262	316	0.55
19	278	326	0.54
53	264	256	0.49
57	259	270	0.51
59	193	240	0.55
62	604	610	0.50
65	401	403	0.50
Mean			0.52
1	98	1619	0.94
5	153	2551	0.94
6	484	2258	0.82
12	77	1621	0.95
56	191	1673	0.90
Mean			0.91
2	65	1584	0.96
4	108	1409	0.93
7	52	2255	0.98
10	100	1757	0.95
13	66	1756	0.96
14	46	1393	0.97
17	49	1862	0.97
18	46	2037	0.98
20	21	2459	0.99
Mean			0.97

* *cn*/+ ♂♂ × *cn bw* ♀♀.

some. When either a low crossover *pr* recombinant is tested against a high crossover *cn* or a low crossover *cn* is tested against a high crossover *pr* recombinant, the result is indistinguishable from the lower crossover valued recombinant when tested against a normal chromosome. When, however, a low crossover *pr* recombinant is tested against a low crossover *cn* recombinant, the result is a higher level of crossing over than either shows against a normal chromosome. Indeed, the level of crossing over here is higher than in heterozygotes for two high crossover recombinants. Thus it may be concluded that (1) the crossover suppression results from heterozygosity for some genetic element in the *SD* region, and (2) homozygosity for this element probably results in a higher than normal crossover rate.

Further evidence supporting conclusion (2) above is obtained from the following experiments. A chromosome carrying the markers, *b en*, and one carrying *b SD en* were made heterozygous with (1) a normal chromosome marked by *cn*,

(2) *R(cn)-3* which is *SD cn*, and (3) *R(cn)-17* which is *SD^{in} cn*. Crossing over between *b* and *cn* and between *cn* and *en* was measured. The results are given in Table 10. For the case of region I *(b—cn)*, heterozygous *SD* combinations show a reduction of about one crossover unit—undoubtedly reflecting the virtual lack of recombination between *pr* and *cn*. The heterozygote for *R(cn)-17* exhibits roughly the control value (or perhaps slightly lower) while the homozygous *SD* combination, *b SD en/R(cn)-3*, exhibits a higher than normal rate. For the case of region II *(cn–en)* the heterozygous *SD* combinations show the reduction to

TABLE 7

Crossing over between pr *and* cn *in female heterozygotes carrying (1)* pr *recombinants from* pr cn/ R(SD)-4 *females and a normal chromosome marked by* cn *and (2)* cn *recombinants, from this same source, and a normal chromosome marked by* pr. *The controls are* pr/cn. *The males, in all cases, are homozygous* pr cn

Constitution of parental female	Phenotype of progeny				Percent recombination
	pr	*cn*	*pr cn*	+	
pr/cn	4183	4120	41	57	1.17
R(pr)-4/ cn	2185	2255	19	17	0.80
R(pr)-5/ cn	4936	4812	32	29	0.62
R(pr)-7/ cn	5185	5110	28	35	0.61
R(pr)-9/ cn	5396	5367	37	27	0.59
R(pr)-61/ cn	5318	4867	27	30	0.56
R(pr)-64/ cn	4839	4791	37	39	0.78
Mean					0.66
R(pr)-1/ cn	5845	6243	11	16	0.22
R(pr)-3/ cn	4483	4518	8	6	0.16
R(pr)-11/ cn	5233	5280	8	9	0.16
R(pr)-50/ cn	5459	5804	6	4	0.09
R(pr)-52/ cn	5324	5382	11	8	0.18
R(pr)-54/ cn	3481	3505	4	6	0.14
Mean					0.16
pr/ R(cn)-5	4945	6817	50	25	0.63
pr/ R(cn)-12	5732	6708	35	28	0.50
pr/ R(cn)-56	1578	1653	7	15	0.68
Mean					0.60
pr/ R(cn)-2	2955	2987	23	22	0.75
pr/ R(cn)-4	6223	6459	46	49	0.74
pr/ R(cn)-7	6322	6648	51	43	0.72
pr/ R(cn)-13	3527	3629	18	16	0.47
pr/ R(cn)-14	4046	4498	30	35	0.76
pr/ R(cn)-17	6040	6081	33	33	0.54
pr/ R(cn)-18	6076	6346	46	44	0.72
Mean					0.67
pr/ R(cn)-3	5114	5414	4	2	0.06
pr/ R(cn)-9	3613	4277	1	2	0.04
pr/ R(cn)-53	3138	3091	4	6	0.16
pr/ R(cn)-57	2266	2598	2	5	0.14
pr/ R(cn)-59	2921	2900	3	7	0.17
pr/ R(cn)-62	2580	2930	4	3	0.13
Mean					0.12

about one half that of the controls as has been observed earlier, while the others show approximately the control value.

Thus the conclusions that the reduction in crossing over in heterozygous *SD* comes about as the result of heterozygosity for an element and that homozygosity for this element yields a higher than normal crossover rate are confirmed here. The fact that the element in question causes a reduction in crossing over when heterozygous, can be separated into partially functioning subunits (that is. all of

TABLE 8

A summary of the number and distinguishable types of recombinants obtained from R(SD)-4/ pr cn *females*

Type of recombinant	Number recovered	Mean distorting action	Mean sensitivity to *SD*	Mean crossover value between *pr* and *cn*
SD^{in} *cn*	9	0.53	0.97	0.67
SD^{sa} *cn*	5	0.59	0.91	0.60
SD cn	11	0.82	0.52	0.12
pr SD^{+}	13	0.55	0.56	0.16
pr SD^{+}	12	0.53	0.75	0.66

TABLE 9

Crossing over between pr *and* cn *in crosses of females carrying* pr *recombinants from* pr cn/ R(SD)-4 *females and* cn *recombinants, from this same source, by* pr cn *males. The control is* pr/cn

Constitution of parental female	Phenotype of progeny *pr*	*cn*	*pr cn*	+	Percent recombination
pr/cn	6349	6090	65	64	1.03
R(pr)-4/ R(cn)-2	6138	6175	31	32	0.51
R(pr)-4/ R(cn)-13	5131	5409	24	31	0.52
R(pr)-4/ R(cn)-56	5357	5228	38	21	0.55
R(pr)-5/ R(cn)-14	4677	5006	25	17	0.43
R(pr)-61/ R(cn)-14	3726	4355	19	19	0.47
Mean					0.50
R(pr)-4/ R(cn)-57	2749	2727	3	3	0.11
R(pr)-4/ R(cn)-62	2491	2568	3	4	0.14
R(pr)-5/ R(cn)-9	4647	4675	2	2	0.04
R(pr)-61/ R(cn)-9	4982	4962	8	7	0.15
Mean					0.11
R(pr)-54/ R(cn)-2	4301	4788	5	2	0.08
R(pr)-54/ R(cn)-13	3141	3568	2	6	0.12
R(pr)-54/ R(cn)-56	3313	3361	9	5	0.21
Mean					0.14
R(pr)-1/ R(cn)-3	5176	5442	43	39	0.77
R(pr)-50/ R(cn)-59	5450	5670	80	40	1.07
R(pr)-52/ R(cn)-9	5225	5352	58	39	0.91
R(pr)-54/ R(cn)-57	3462	3486	27	29	0.80
R(pr)-54/ R(cn)-62	5066	5491	56	31	0.82
Mean					0.87

the recombinants show some depression in crossing over), and that homozygosity for the element yields a higher than normal crossover rate suggests—but by no means proves—that the element in question is an insertion or duplication of genetic material in the *SD* region.

Reconstitution of SD: Since it is clear that *SD* is not a simple locus, it seemed possible that a functioning *SD* might be reconstituted in recombinants between *R(cn)* and *R(pr)*. A series of such re-recombinants were obtained by collecting wild-type chromosomes from *R(pr)/R(cn)* heterozygous females. Tests for segregation-distortion in such re-recombinants is given in Table 11. It can be seen that only in the one case that the *pr* recombinant was insensitive and the *cn* recombinant SD^{sa} was a functional *SD* recovered. It may be noted, however, that re-recombinants from SD^{sa} give uniformly higher *k* values than re-recombinants from SD^{in} which is additional evidence that SD^{sa} and SD^{in} are distinct.

In addition, about 30 *pr cn* re-recombinants were collected from these same crosses and tested for segregation-distortion. None of these were distorters.

A test of the crossover properties of a sample of wild-type re-recombinants is given in Table 12. It is evident that the reconstituted distorter chromosome exhibits the typical low crossover property, while the nondistorting re-recombinant from the same *pr* recombinant shows the usual intermediate level of recombination. In addition, re-recombinants from a high crossover *pr* recombinant and a high crossover *cn* recombinant show a level of crossing over slightly higher than that of either parent.

Interpretation of results: The following properties of the *SD* region have been elucidated by the experimental data presented here.

1. Crossing over in the immediate vicinity of *SD* is reduced by about a factor of ten.

2. This reduction in recombination is independent of the "state" of *SD* or of SD^{+}.

TABLE 10

Crossing over between b *and* cn *(region I) and between* cn *and* en *(region II) in crosses of females heterozygous for* b en *on one chromosome and* cn *on the other, by* b pr cn en *males.* R(cn)-3 *is* SD; R(cn)-17 *is* SD^{in} *(Table 4)*

Phenotype of progeny	Constitution of parental female					
	b cn/cn	*b SD cn/cn*	*b en/R(cn)-3*	*b SD en/R(cn)-3*	*b en/R(cn)-17*	*b SD en/R(cn)-17*
b en	2414	3978	2270	3017	2434	1731
cn	2964	5231	2699	4318	2853	2761
b cn	121	101	79	200	114	63
en	104	90	70	178	76	43
b	124	96	61	189	106	53
cn en	88	75	49	96	91	41
b cn en	3	3	0	2	4	3
+	4	4	3	5	2	2
Percent crossovers						
Region I	3.98	2.07	2.91	4.81	3.45	2.36
Region II	3.76	1.86	2.16	3.65	3.57	2.11

3. The crossover suppression diminishes rapidly with increasing distance of the measured region from *SD*.

4. The effect on crossing over is confined to the right arm of chromosome II.

5. The reduction in crossing over comes about as a consequence of heterozygosity for an element.

TABLE 11

Tests for segregation-distortion by wild-type re-recombinants from females heterozygous for nondistorting pr *recombinants and* cn *recombinants that are either* SD^{in} *or* SD^{sa}. *The cross is of males, heterozygous for the wild-type re-recombinant and* cn bw, *by* cn bw *females*

Recombinant	*cn bw*	+	*k*	Recombinant	*cn bw*	+	*k*
R(pr-4/cn-2)	432	503	0.54	*R(pr-61/cn-14)*	660	784	0.54
	529	561	0.51		918	1100	0.55
	781	875	0.53		456	491	0.52
	488	506	0.51		584	663	0.53
	684	773	0.53		685	700	0.51
	694	744	0.52		689	810	0.54
	546	604	0.53	Mean			0.52
	772	930	0.55				
	795	798	0.50	*R(pr-4/cn-56)*	545	674	0.55
	635	631	0.50		596	649	0.52
	904	913	0.50		743	867	0.54
	674	718	0.52		864	998	0.54
	669	716	0.52		518	671	0.56
					709	903	0.56
R(pr-4/cn-13)	477	517	0.52				
	688	732	0.52	*R(pr-5/cn-1)*	680	762	0.53
	662	685	0.51	*R(pr-5/cn-5)*	653	750	0.53
	667	734	0.52		543	612	0.53
	620	707	0.53		116	117	0.50
	747	827	0.53		256	331	0.56
	602	584	0.49				
	865	891	0.51	*R(pr-5/cn-12)*	414	514	0.55
	782	816	0.51		599	792	0.57
*	604	650	0.52				
	743	742	0.50	*R(pr-61/cn-5)*	563	752	0.57
	855	964	0.53	*R(pr-61/cn-12)-2**	955	1497	0.61
	629	621	0.50	*-3**	1168	1475	0.56
				*-4**	912	1377	0.60
R(pr-5/cn-14)	773	771	0.50		259	447	0.63
	415	460	0.53				
	818	803	0.50	Mean			0.56
	759	919	0.55				
				R(pr-52/cn-4)	875	1029	0.54
				R(pr-54/cn-13)	1170	1208	0.51
				*	1086	1282	0.54
					1479	1529	0.51
					1380	1442	0.51
				Mean			0.52
				R(pr-54/cn-56)	260	1260	0.83

TABLE 12

Crossing over between pr *and* cn *in females, heterozygous for* pr cn *and wild-type recombinants from females carrying* pr *recombinants and* cn *recombinants from* pr cn/R(SD)-4 *females by* pr cn *males. The lines used are those marked by an asterisk in Table 2*

Recombinant	Phenotype of progeny				Percent recombination
	pr cn	+	*pr*	*cn*	
R(pr-54/cn-13)	5339	5701	35	28	0.57
R(pr-4/cn-13)	4856	5563	34	20	0.52
R(pr-54/cn-56)	5260	5807	7	6	0.12
R(pr-61/cn-12)-2	3916	3982	35	34	0.87
R(pr-61/cn-12)-3	4191	4864	44	38	0.90
R(pr-61/cn-12)-4	4191	4460	43	22	0.75

6. Homozygosity for this element results in a rate of recombination somewhat higher than normal.

7. The properties of the three types of *cn* recombinant and two kinds of *pr* recombinant that are recovered from crossing over in the *SD* region have been tabulated in Table 8. It should be noted that in no instance is crossing over normal, nor are any completely normal *SD*-bearing chromosomes recovered.

8. From heterozygotes for a nondistorting, insensitive *pr* recombinant and a semiactive, somewhat insensitive *cn* recombinant, an active, completely insensitive, low crossing over *SD*-bearing chromosome has been recovered.

Since there is nothing in these data to suggest otherwise, we assume that they are formally interpretable in terms of a linear sequence of elements. It is, of course, possible to construct any number of models based on other assumptions, but this does not seem profitable at this time. Thus we assume that closely linked, and to the right of *SD* itself, there is an Activator of *SD*, *Ac(SD)*, that is necessary in order that *SD* function. On this interpretation SD^{in} is *SD* alone while SD^{sa} is *SD Ac(SD)*. These two elements alone, however, do not account for the recombination results, nor can we understand why *SD Ac(SD)* is such a weakly distorting *SD* chromosome nor why the *SD cn* recombinants are weaker distorters than the original *SD* line. To account for these facts we may suppose the *SD* region to be an aberration (and, for the reasons given above, possibly an insertion or duplication of genetic material) in which *SD* and *Ac(SD)* reside. All of the exchanges in the *SD* region apparently occur within this aberration which acts cumulatively on crossing over so that a part of the aberration yields the intermediate level of crossing over while a majority or all of the aberration results in the low level of exchange. Supposing the aberration to be an insertion accounts for the fact that (1) it acts more or less additively in crossing over, (2) that it reduces crossing over by virtue of heterozygosity and (3) that homozygosity for the region results in a higher than normal rate of exchange. The aberration, however, must also have the effect of enhancing segregation-distortion and suppressing sensitivity. This point will be developed below.

A schematic representation of the postulated *SD* region and the exchanges that can take place within it is shown in Figure 1. Exchange "a" produces an insensi-

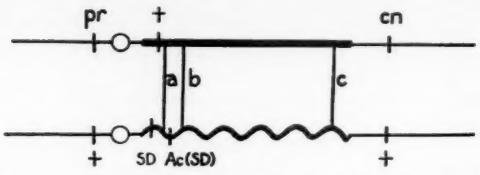

FIGURE 1.—A schematic representation of the *SD* region. The indicated crossovers are those that occur between the markers *pr* and *cn*. The heavy lines represent homologous sections in the *SD* region. The wavy line is the aberrant region responsible for the reduction in crossing over and for insensitivity of the original *SD*-bearing chromosome.

tive *pr* recombinant and an SD^{in} *cn* recombinant; exchange "b" produces insensitive *pr* and SD^{sa} *cn* recombinants; and exchange "c" yields a semisensitive *pr* and *SD cn* recombinant.

On the action of the aberration: From the foregoing, the effects of the aberration on crossing over are understandable. It is necessary, however, to postulate, in addition, effects on the phenomenon of segregation-distortion itself. In particular, the aberration evidently enhances the action of *SD* since the original *SD* line, containing the aberrant region intact, produces higher k values than the recombinant *SD cn* lines which have lost some of it, and these produce higher k values than the SD^{sa} recombinants which have lost most of the aberrant region. A second effect of the aberration is that it imparts insensitivity to chromosomes carrying it since the *pr* recombinants that show very reduced crossing over are insensitive to a standard *SD*, and those that exhibit an intermediate level of crossing over are more sensitive. Similarly, the original *SD* chromosome and the *cn* recombinants that show low crossing over are insensitive, and those that carry *SD Ac(SD)*, while much more sensitive than these, are less sensitive than those that carry only *SD*.

To explain these results we make three assumptions based on the prior formalism presented by SANDLER, HIRAIZUMI and SANDLER (1959) and summarized in Proposition II. (1) An activated *SD* chromosome will cause a distorting misreplication in either itself or its homolog—but not both in the same meiosis. (2) The misreplication always occurs in the region of the aberration (the heavy line in Figure 1). (3) The aberrant region (the wavy line in Figure 1) never misreplicates. These three assumptions account for all of the observations thus far in the following way. First, *SD* normally shows a high k value owing to the fact that *SD* carries the aberration and therefore always affects its homolog rather than itself. Second, *SD* is completely insensitive to the action of another *SD* because it carries the aberrant region which cannot be affected by *SD*. Third, the *SD cn* recombinants are weaker distorters than the original lines because when any section of the aberration is missing from an *SD Ac(SD)*-bearing chromosome, the k value goes down owing to some proportion of misreplication of the *SD*-bearing chromosome itself instead of the homolog. Fourth, the presence of the aberrant region, or any part of it, on the homolog in an *SD* heterozygote will imply a degree of insensitivity of the homolog in proportion to the amount of the aberrant section

that it carries owing to the immunity of the aberration to the action of *SD*. Finally, the fact that the SD^{sa} recombinant shows any abnormal segregation at all is accounted for by its carrying a small part of the abnormal region (Figure 1) which implies a slightly higher probability of the homolog misreplicating than *SD* acting on itself.

A test of this general hypothesis suggests itself. If a *pr* recombinant carrying most of the abnormal region (i.e., an insensitive, low crossover *pr* recombinants) is made heterozygous with an *SD Ac(SD)*-bearing *cn* recombinant in males, then a distorted segregation ratio *against* the *SD*-bearing chromosome should result. Accordingly, *R(cn)-1* which is SD^{sa} and *R(cn)-7* which is SD^{in} were made heterozygous with a number of *pr* recombinants in males, and the segregation ratios measured. These results are given in Table 13. It can be seen that when the *SD*-bearing chromosome does not carry *Ac(SD)* (i.e., *R(cn)-7*), there is no distorted segregation. Likewise, when the *pr* recombinant does not carry most of the aberration (i.e., semisensitive *pr* recombinants), there is no distorted segregation irrespective of the *cn* recombinant. When, however, *SD Ac(SD)* is heterozygous with a *pr* recombinant carrying most of the aberration, a distorted segregation ratio favoring the *pr* recombinant is evident. Not only are the mean *k* values different from 0.50, but in every test individual cultures gave high *k* values (greater than 0.80).

TABLE 13

The results from crosses of males, heterozygous for various R(pr)/R(cn) *combinations, by* cn bw *females. The* k *value given in the last column is the proportion of* R(pr) *progeny recovered*

Constitution of parental male	Phenotype of progeny		
	cn	+	*k*
pr-4/cn-1	1479	1467	0.50
pr-64/cn-1	3555	3474	0.49
Mean			0.50
pr-4/cn-7	1579	1789	0.53
pr-64/cn-7	1869	1892	0.50
Mean			0.52
pr-1/cn-1	2163	3880	0.64
pr-2/cn-1	1728	2468	0.59
pr-3/cn-1	1852	2922	0.61
pr-6/cn-1	2399	4471	0.65
pr-11/cn-1	1489	3448	0.70
pr-50/cn-1	1140	2201	0.66
pr-52/cn-1	2485	4092	0.62
Mean			0.64
pr-1/cn-7	2826	3391	0.55
pr-2/cn-7	2370	2552	0.52
pr-3/cn-7	2220	2619	0.54
pr-6/cn-7	2723	2844	0.51
pr-11/cn-7	2210	2719	0.55
pr-50/cn-7	1375	1538	0.53
pr-52/cn-7	1399	1537	0.52
Mean			0.53

The *pr* recombinants should be of two types: those carrying *Ac(SD)* and those lacking it (Figure 1). There do indeed appear to be differences among lines in these experiments in respect of the amount of distortion of the segregation ratios. These differences may be due to whether or not a *pr* recombinant carries *Ac(SD)*—and if it does it may result in either a higher or lower *k* value than if it does not. The differences may, on the other hand, be continuous reflecting the amount of the aberrant region that any *pr* recombinant happens to carry.

Finally, it ought to be noted that since no distortion is evident in any case in which the *SD*-bearing chromosome does not carry *Ac(SD)*, even though some *pr* recombinants should carry *Ac(SD)*, it would appear necessary to suppose that *Ac(SD)* must be in coupling with *SD* in order to activate it.

DISCUSSION

The results and interpretation presented in this report indicate that the *SD* region is extremely complex. The notion of an Activator of *SD*, necessary in order that *SD* act, coupled with the prior notion that *SD* causes something formally equivalent to a chromosome break in its homolog (SANDLER, HIRAIZUMI and SANDLER 1959), strongly suggests analogy with the "*Ac–Ds*-like" systems in maize (MCCLINTOCK 1951, 1956). Indeed, the possibility indicated above, that an extra *Ac(SD)* might inhibit *SD* action would be very suggestive of the action of *Ac* in maize. In the maize systems, however, no obvious analogy with the immune aspect of the aberration exists—this element seems unique to the *SD* system.

It should be emphasized, finally, that the notion that the aberration is an insertion or duplication and the crossover model presented are merely formalisms; the true structure of the *SD* region may cytologically be quite different from what is inferred genetically. For example, the question of how the aberration shares homology with the corresponding section on the normal homolog is unresolved because the exchanges in this region between *SD* and a normal chromosome might well be occurring in the heterochromatin in which the rules of homology and crossing over are extremely complex and nonspecific (for example LINDSLEY 1955).

Evidence has been presented previously (SANDLER, HIRAIZUMI and SANDLER 1959) indicating that synapsis in the *SD* region is necessary in order that segregation-distortion occur. In particular, if structurally abnormal SD^+-bearing second chromosomes are used as homologs for *SD*, distortion is suppressed or eliminated entirely. For example, *In(2LR)Cy/SD* males uniformly produce *k* values of approximately 0.50. This inversion has a break point in the centromere region, and thus presumably interferes with synapsis in the *SD* region.

From a female heterozygous for *In(2LR)Cy* and *cn bw*, a *Cy cn bw* chromosome was recovered, which proved, upon testing, to carry *In(2L)Cy* but no inversion on the right arm which is now marked by *cn bw*. Furthermore, crosses of *In(2L)Cy cn bw/Ty* females by *cn bw* males yielded 578 *Cy cn bw;* 480 *Cy cn;* 12 *Cy bw;* 12 *Cy;* 14 *cn bw;* 4 *cn;* 586 *bw;* and 689 + progeny. In this cross,

therefore, crossing over between the right break point of *In(2L)Cy* and *cn* (which must be roughly equivalent to crossing over between the centromere and *cn*) is 1.8 percent which is approximately that observed in the *pr cn* region. Thus it appears that synapsis in *In(2L)Cy* heterozygotes is normal in the *SD* region and therefore, under the hypothesis that synapsis is required for distortion, the *In(2L)Cy cn bw* chromosome ought to be sensitive.

Accordingly, *SD-5/ cn bw* males were crossed to *In(2L)Cy cn bw/cn bw* females, and in the F_1 *SD-5/cn bw* and *SD-5/ In(2L)Cy cn bw* males were collected and crossed to *cn bw* females. In the control cross (*SD-5/cn bw* males), 137 *cn bw* and 4,197 + progeny were recovered giving a control $k = 0.97$. In the experimental cross (*SD-5/In(2L)Cy* males), 188 *Cy cn bw* and 3,879 + progeny were produced giving an experimental $k = 0.95$. Thus it is clear that the *In(2L)Cy* chromosome is approximately as sensitive as the standard *cn bw* chromosome.

The fact that *In(2LR)Cy*, in which synapsis in the *SD* region is disturbed, is not sensitive, whereas *In(2L)Cy*, in which synapsis is normal near *SD*, is sensitive, provides additional evidence that synapsis in the *SD* region is related to the phenomenon of segregation-distortion. This argument, of course, is subject to the assumption that the crossover results in the female may be used as an indicator of the synaptic situation in the male.

It is now clear, however, that synapsis in the *SD* region is always abnormal owing to the presence of the crossover suppressing aberration in the *SD* region. These results may, therefore, be most simply interpreted as meaning that it is not just intimate synapsis that is prerequisite for distortion—but, rather, what is required is some special synaptic condition that is disturbed by a break point in the immediate vicinity of *SD*. Perhaps a part of the reduction in distortion that accompanies the loss of a section of the abnormal region is due to a breakdown of the optimum synaptic condition.

The results presented here explain the behavior of an exceptional *SD*-bearing recombinant reported earlier (SANDLER and HIRAIZUMI 1960). It had been shown that at the tip of IIR, closely linked to bw^+, there is a Stabilizer of *SD*, *St(SD)*, in the presence of which *SD* is stable and in the absence of which *SD* is less stable (i.e., exhibiting considerable variation in *k* values from male to male). Thus all recombinants between the locus of *cn* and the locus of *bw* in *SD/cn bw* heterozygotes lose *St(SD)* and are therefore less stable than the original *SD* line. However, one recombinant, *R(SD-5)-26*, appeared to result from a double crossover, one exchange between *SD* and *cn* and the other between *bw* and an inversion break point whose relative position is known. This recombinant should carry *St(SD)* but, in fact, showed a very low *k* value (mean $k = 0.76$). It is now clear that this recombinant, in which an exchange occurred between *SD* and *cn* lost a part of the aberration, since all recombination in the *SD* region occurs within the abnormal region, and this undoubtedly accounts for the low *k* value.

It has been noted previously (SANDLER and HIRAIZUMI 1959) that structurally normal, SD^+-bearing second chromosomes differ, one from the other, in their sensitivity to *SD* (i.e., in the characteristic *k* value distribution observed when

they are tested against some standard *SD* line). The data presented here on the substructure of the *SD* region provide evidence on the exact location of the differences among SD^+ lines of different sensitivities.

It should be noted in the first place that insensitivity of SD^+ chromosomes almost certainly comes about by a mechanism different from the insensitivity imparted by the aberration carried by SD-bearing chromosomes. The argument for this point will be presented in detail in a subsequent communication but in essence it is that insensitivity of SD^+ chromosomes comes about by an inactivation of the *SD* allele. The evidence here is that the action of an *SD* chromosome is impaired by heterozygosity with an insensitive SD^+ allele for some generations after the insensitive allele has been removed from the system ("translocal modification"; SANDLER and HIRAIZUMI 1959). The insensitivity of the aberration, on the other hand, apparently comes about by virtue of a nonreactivity of the aberration-bearing homolog of *SD* itself, since otherwise *SD* should not have acted on itself as in the experiments described above.

Furthermore, the sensitivities exhibited by the various classes of *pr* recombinants and *cn* recombinants almost certainly imply that differences in sensitivity of SD^+-bearing chromosomes are localized at the *SD* locus precisely. This is so for the following reasons. The insensitive region of SD^+-bearing chromosomes is known to be generally in the centromere region (SANDLER and HIRAIZUMI 1959). The sensitivity of this region from the *pr cn* second chromosome (that chromosome from which the *pr* and *cn* recombinants were derived) backcrossed to *cn bw* for many generations (to make the chromosome comparable to the recombinants) was tested in a cross of *SD*/*pr cn* males by *cn bw* females. The progeny included 1,567 *cn* and 6,090 + flies; thus the average k value was 0.80. This is very similar to the sensitivity of the semisensitive *pr* recombinants (Table 8). Thus those *pr* recombinants that contain just the SD^+ element from *pr cn* exhibit the sensitivity of *pr cn*. Much more convincing, however, is the fact that those *cn* recombinants that are SD^{in}, and therefore contain all of the *pr cn* centromere region except the SD^+ element, are completely sensitive. These observations certainly suggest strongly that the relative insensitivity of *pr cn* is located precisely at the SD^+ element.

Finally, it is perhaps worth noting that these data provide additional evidence that the *SD* system has existed in nature for a long time, for it must, indeed, have required a long time for the complexity of the *SD* region indicated by these studies to have evolved.

SUMMARY

The segregation-distorter locus *(SD)* is in the centromere region of chromosome II. It has been found that the *SD* region is genetically complex. The data collected may be interpreted in the following way. The *SD* locus itself causes a misreplication (formally equivalent to a break of some sort) either in its immediate vicinity or in a comparable region of its homolog. Closely linked and to the right of *SD* there is an Activator of *SD*, *Ac(SD)*, which must be present in coupling with *SD* in order that *SD* function. Both *SD* and *Ac(SD)* are located within

a small chromosomal aberration (which can be thought of formally as a duplication or insertion) that has the property of being immune to the action of the *SD Ac(SD)* complex. Thus the aberration results in (a) *SD* being immune to its own action and (b) the *SD* chromosome always acting on its homolog.

It has been possible to separate the elements in the *SD* region by crossing over and also to reconstitute *SD* from its constituent parts by further recombination. In addition, by separating appropriate parts of the *SD* region and making them mutually heterozygous in males, a system in which the *SD*-bearing chromosome acted on itself instead of its homolog was found.

The exact evidence bearing on this interpretation of the *SD* region is presented and the implications of this complexity discussed.

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LITERATURE CITED

LINDSLEY, D. L., 1955 Spermatogonial exchanges between the X and Y chromosomes of *Drosophila melanogaster*. Genetics **40**: 24–44.

MCCLINTOCK, BARBARA, 1951 Chromosome organization and genic expression. Cold Spring Harbor Symposia Quant. Biol. **16**: 13–47.

1956 Controlling elements and the gene. Cold Spring Harbor Symposia Quant. Biol. **21**: 197–216.

SANDLER, L., YUICHIRO HIRAIZUMI, and IRIS SANDLER, 1959 Meiotic drive in natural population of *Drosophila melanogaster*. I. The cytogenetic basis of Segregation-distortion. Genetics **44**: 233–250.

SANDLER, L., and YUICHIRO HIRAIZUMI, 1959 Meiotic drive in natural populations of *Drosophila melanogaster*. II. Genetic variation at the *Segregation-distorter* locus. Proc. Natl. Acad. Sci. U.S. **45**: 1412–1422.

1960 Meiotic drive in natural populations of *Drosophila melanogaster*. IV. Instability at the Segregation-distorter locus. Genetics **45**: 1269–1287.

ALPHA AND BETA LOCI IN DROSOPHILA

C. AUERBACH AND BARNET WOOLF

Institute of Animal Genetics, Edinburgh

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IN a recent paper (1959a) FAHMY and FAHMY claim that the Drosophila genome consists of "two classes of genes, which differ in their stability and manner of response to mutagens."

These they call alpha and beta loci. They further claim to have established a differential response to mutagens within the beta, but not within the alpha, class, and they consider this "the most interesting recent development in mutagenesis studies." It is worth-while to examine the evidence brought forward in favour of these claims.

MATERIAL

All mutants used in the FAHMYS' investigation were sex-linked.

The 43 *alpha mutants* are said to have been "previously detected in radiation mutagenesis work." This, however, is true for only 27 of them, and of these 24 have also occurred spontaneously. The remaining 16, according to BRIDGES and BREHME (1944), have only occurred spontaneously.

The 63 mutants used were chosen from several hundred "new mutants" at "hitherto undescribed loci which have mutated in our laboratory mainly by chemical means." The following among them do not fulfill these conditions of choice.

small-wing, of which three occurrences are listed in BRIDGES and BREHME, one by radiation;

Turned-up, which is now admitted by the FAHMYS to have occurred before;

scute^{fahmy} at 0.0, reported to be a deficiency of bands 1B1-2, viable in hemizygotes and inviable in homozygotes, and giving a near-normal phenotype in heterozygotes with scute;

notchoid, a viable allele of Notch, as is easily verified by crossing it to a Notch deficiency;

bronzy, an allele of the radiation induced maroon-like (GLASSMAN 1959). Phenotypically, bronzy is subject to a maternal effect: hemizygotes and homozygotes are wild-type when the mother was so. In old cultures, the maternal effect disappears. In Muller-5 tests (the source of all beta mutants) bronzy must therefore always have seemed to occur as a gonadic mosaic; curiously enough, this

Editorial Note: This article is a criticism of an article that was published in GENETICS. The subject is of such importance that the Editors made an exception to the policy that manuscripts must report an author's research. An acceptable criticism of an article accepted for publication by the Editors should appear in the same journal.

has not been mentioned or observed by the FAHMYS. The maternal effect will be found to play a rôle in the interpretation of the experimental data.

It is true that the FAHMYS never make it quite clear whether they restrict the term beta mutations to those that occur at new loci, or whether they include new alleles at known loci. In the latter case, notchoid, scute^{fahmy}, and bronzy might qualify as beta mutants. The possibility of allele rather than locus specificity in response to mutagens is, indeed, interesting. It seems to find support in a recent preliminary communication by the FAHMYS (1959b) in which they intimate that different cistrons of the rudimentary locus respond differently to mutagens. The rudimentary locus is one of the alpha loci chosen for the experiments under discussion. If allele specificity were used for classification, an unknown proportion of mutations at this locus should, therefore, be put into the beta class together with bronzy and notchoid; the same presumably applies to the other alpha loci. Since classification into alpha and beta *alleles* is not generally possible, it cannot legitimately be applied to the few cases in which chemical treatment produced alleles with new phenotypical features.

In the discussion of the FAHMYS' paper, more criteria are added to the definition of beta loci.

(1) Beta mutants "are on the whole markedly more pleiotropic in their phenotypic manifestations" than alpha loci. In this respect, the ten beta loci chosen for extended studies are atypical; for with the exception of *Oce*, none is reported to have pleiotropic effects (FAHMY and FAHMY 1958a).

(2) Many beta mutants "are slow in development and, therefore, late in eclosion and also have reduced viability and/or fertility." Again, the ten chosen beta loci are atypical, for viability and fertility are stated to be "good" or "excellent" in all of them, and delayed emergence is noted for only one *(tc)* (FAHMY and FAHMY 1958a).

(3) Beta loci are more "radioresistant" than alpha loci. Proof of this statement forms the main subject of the present investigation and will be examined below. It is not clear how important it is for identification of beta loci. The mutant small-wing was not promoted to the alpha-class when its occurrence after irradiation was pointed out to the FAHMYS. Conversely, when smalloid, a beta mutant, appeared even more frequently after X-radiation than after chemical treatment, it was called a "radiation responsive locus which has been missed by earlier workers." Whether this turns it into an alpha locus is not clear; it remains included in the calculations on the first set of tests, but is removed from the second tests.

(4) Beta loci "are on the whole less responsive to mutagens than the alpha class." This is claimed to be shown in the present publication. Even if it were true for the particular sample used in these experiments, it can hardly be maintained as a general statement. Many of the mutants listed in BRIDGES and BREHME have been reported only once, and even in the experiments by the FAHMYS many beta mutants occurred more often than rare alpha ones.

(5) The great majority of beta loci respond differentially to various chemical mutagens. This, too, is claimed to be shown in the present publication and will be

discussed below. It may be said here that even if the results justified this claim—and they do not—heterogeneity in respect to mutagens has been tested only for a whole *group* of beta loci; whether any single beta locus shows differential response to mutagens has not been tested and would, indeed, be difficult to establish.

Altogether, the definition of beta loci is hazy and arbitrary in the extreme. The only criterion that is fulfilled by all beta mutants used in the present experiments is that they have been found by the FAHMYS in tests with chemicals and that, at the time of their discovery, the FAHMYS believed they had not occurred before.

METHODS

The data used in the first part of the FAHMYS' investigation were obtained in Muller-5 tests for sex-linked lethals; in the second part, specific-loci tests were carried out.

Muller-5 tests: These were carried out in the usual way. Visible mutants were scored in the F_2 cultures; in order to reduce personal bias to a minimum, scoring was done exclusively by M. J. FAHMY. The method of scoring has never been fully described. Scoring, under a low-power binocular, of unetherized cultures, as practiced by mutation workers in general and by the FAHMYS originally (1955), can hardly be a reliable method for the detection of the many mutants that are listed as "not easily classified" (FAHMY and FAHMY 1958). Moreover, scoring of cultures "rather early after emergence" (FAHMY and FAHMY 1955) would not have detected bronzy or any of the late-emerging mutants, unless these are at the same time semilethal. The latter class was detected because the FAHMYS, in contrast to most mutation workers, preserve all suspected lethal cultures until no more flies emerge. A remark on p. 1157 (FAHMY and FAHMY 1959a) throws light on the technique employed for scoring. It is stated there that the early experiments with TEM (triethylenemelamine) and the carboxylic mustards could not be used as sources of beta loci because they had been undertaken "before the accumulation of the beta loci." Since these same experiments were considered unobjectionable sources of alpha loci, this indicates a change in scoring technique between the early experiments and the later ones. If this is so, it explains why the ratio of visible to lethal mutations was unusually high in all recent experiments by the FAHMYS, but not in their early ones.

The Muller-5 tests that served as sources of alpha and beta mutants were carried out with different, unspecified doses. It is assumed that the proportion of different types of mutants is not affected by dose. This is probably a correct guess; its validity is, however, not improved by the statement that "there is no evidence for a threshold effect for particular loci". Evidence of this type would, indeed, be difficult to obtain, and this has never been attempted. The mutants were also produced in germ cells of different, unspecified stages. Here, the assumption that this can play no rôle in creating an apparent specificity seems much less justified. It is entirely conceivable that mutations in early spermatogonia may tend to be different in kind from those induced in spermatozoa. It might, e.g.,

not be an accident that three out of four mutations to slim occurred among the 25 beta mutants induced by CB1506 (chloroethyl methanesulphonate), which acts almost exclusively on spermatogonia.

Specific loci tests: Wild-type males were treated with either X-rays or an amino-acid mustard (AAM) and mated to females that were homozygous or heterozygous for genes at loci whose mutability after these two treatments was to be tested. The F_1 flies were examined for mutant phenotypes. Mutants were submitted to further tests in order to distinguish between lethal and viable ones. The former were attributed to deficiencies. Cytological checks were, apparently, not carried out, so that the possibility of lethal point mutations (such as have, e.g., occurred quite frequently at the cut locus) is neglected.

Scoring in these experiments was apparently not done exclusively by M. J. FAHMY, and indeed the experiments were carried out on such a large scale that it would hardly have been possible for one person to score them unaidedly. It is, however, regrettable that this was so; for while specific-loci tests for easily classifiable mutants can safely be carried out by several workers in cooperation, this is not so in the present case. One of the ten beta mutants used, concave wing, is listed in DIS (1958a) with the remark "not easily classified". For three others, ruddle, copper, and bronzy (all slight eye colour deviations towards a brown tinge) trickiness of classification is implied by the remark "best classification in newly emerged flies". The mutant smalloid is given Rank 2 in spite of good viability and fertility, presumably because in crowded cultures it is difficult to distinguish a genotypically small from an undernourished fly.

For a specific-loci test to provide valid data on mutagen specificity, certain general conditions have to be fulfilled. These are:

(1) The two kinds of treatment must be of comparable strength. In Drosophila, this can be achieved by choosing treatments that yield comparable frequencies of sex-linked lethals.

(2) Culture conditions must be strictly standardized both within and between series. This is particularly necessary when late-emerging mutants (like *tc*) are scored.

(3) Care must be taken to avoid—or correct for—bunching and germinal selection, which may occur when premeiotic cells have been treated.

(4) The same loci must be tested with both mutagens.

(5) The scale of the experiments should be chosen in advance so as to give either comparable numbers of progeny after each treatment or a larger number after the treatment expected to yield fewer mutants.

Of these prerequisites, only (1) has definitely been taken care of by the FAHMYS. Indeed, they have carried accuracy to an extraordinary degree. In every experiment with AAM, an aliquot of flies was tested for sex-linked lethals, and the dose-equivalent was calculated in units of r. Since three percent sex-linked lethals are roughly equivalent to 1000r, this implies that the determination of lethal frequencies in these tests was accurate within less than .01 percent. In actual fact, it is doubtful whether any of the dose measurements—for radiation as well as chemical treatment—is accurate within 1000r. Progeny for the lethal

tests was sampled during the nine days following treatment. During this period, the frequency of X-ray induced lethals varies by a factor of two or more (AUERBACH 1954); for AAM FAHMY and FAHMY (1958b) have shown the existence of a similar brood difference. If precautions were taken to minimize this variation, they are not described.

(2) Whether or not precautions were taken to standardize culture conditions, they are not described. In particular, the authors do not explain what steps were taken to avoid as far as possible fluctuations of population density between bottles belonging to different experiments, or to different series of the same experiment.

(3) Errors due to bunching or germinal selection are said to have been avoided by testing only progeny obtained during the first nine days after treatment because "under our experimental conditions" samples of spermatozoa collected during this period are presumed to stem entirely from treated postmeiotic stages. One wonders what these experimental conditions were; they are not described. In their previous investigations on brood patterns, the FAHMYS have adhered to a standard breeding procedure, giving each male one new female every three days. It is possible that their claim is justified for this slow-breeding procedure, although experiments recently carried out in this Institute cast doubt on it (REDDI, in press). In any case, it seems unbelievable that this procedure should have been used in experiments of the magnitude described here. If it was not used, where is the proof that progeny produced during the later days of sampling did not come from treated meiotic or premeiotic cells?

The remaining conditions were definitely not fulfilled. This can be inferred from Table 1, in which Tables 4 and 5 of the FAHMYS' (1959a) paper have been rearranged in such a way that the actual experiments can be reconstructed. In particular, this table makes it possible to infer what strains of females were used as testers. While it is just conceivable that two X-ray experiments should have been carried out with exactly the same dose, it is inconceivable that two experiments with AAM should have given exactly the same dose equivalent. It appears that the following tester chromosomes were used:

I. *sc ct wy g f* (X-rays 2990r)

II. *ccw tc sl bz* (X-rays 2990r; AAM 2563 "r")

Presumably, the females in the radiation test were heterozygous for I and II. If so, this rules out bronzy as scorable mutant, since it would be masked by the maternal effect of the phenotypically wild-type P_1 females. It is just possible that, instead, two homozygous tester stocks were used in the two X-ray experiments with 2990r. This is, in fact, suggested by the different numbers of progeny counted. Even so, however, bronzy will have to be disqualified because it could not be scored in the experiment with AAM, in which the tester females definitely were heterozygous (see below).

III. *v* (X-rays, 3207r; AAM 2563 "r")

In the AAM experiment with 2563 "r", chromosomes II and III must have been combined in heterozygotes.

IV. *brc Oce cop* (X-rays 3834r)

V. *omm* (X-rays 3834r; AAM 3125 "r")

TABLE 1

Data of Tables 4 and 5 in the paper by FAHMY *and* FAHMY *rearranged for the sake of better comparison*

Locus α	Locus β	X-rays Dose (r)	X-rays Chromosomes	X-rays No.	X-rays Mutants Leth.	X-rays Mutants Viable	Mustard Dose equivalent "r"	Mustard Chromosomes	Mustard No.	Mustard Mutants Leth.	Mustard Mutants Viable
sc		2990	I/I*	38,352	2	4					
ct		2990	I/I*	38,352	6	1					
wy		2990	I/I*	38,352	7	4					
g		2990	I/I*	38,352	6	5					
f		2990	I/I*	38,352	1	3					
	ccw	2990	II/II*	44,071	2	0	2563	II/III	87,547	0	1
	tc	2990	II/II*	44,071	0	0	2563	II/III	48,977	0	1
	sl	2990	II/II*	44,071	0	0	2563	II/III	74,253	0	6
	bz	2990	II/II*	44,071	8	0	2563	II/III	47,036	1	2
v		3207	III/X	89,324	2	4	2563	II/III	129,831	2	3
	up	3207	III/X	61,422	2	0					
	omm	3834	IV/V	28,626	2	0					
	brc	3834	IV/V	28,626	0	0	3152	IV/VI	67,445	0	1
	Oce	3834	IV/V	28,626	2	0	3152	IV/VI	67,445	1	1
	cop	3834	IV/V	28,626	0	0	3152	IV/VI	67,445	2	1
	nd	4266	VI/VI	34,664	0	0	3152	IV/VI	67,445	12	1
	smd	4266	VI/VI	22,278	1	8	3152	IV/VI	67,445	1	8
	rud	3418	VII/VIII	47,272	2	0	3465	VIII/VIII	44,051	0	4
	sma	3418	VII/VIII	47,272	0	0					
	Tu	3418	VII/VIII	47,272	0	0					
car		3418	VII/VIII	66,978	7	0					
	mo	2678	IX/IX	58,833	2	0					
	mel	2777	XI/XI	32,867	0	0					

* or I/II.

Chromosomes IV and V were used in heterozygous conditions for the X-ray test with 3834r. In the AAM test with 3125 "r", chromosome V was used in heterozygous condition with

VI. *nd smd* (AAM 3125 "r")

VII. *sma Tu car* (X-rays 3418r)

VIII. *rud* (X-rays 3418r; AAM 3465 "r")

Chromosomes VII and VIII were used in heterozygous condition for the X-ray test with 3418r. Chromosome VIII by itself was used for the AAM test with 3465 "r".

IX. *mo* (X-ray 2678r), presumably used in homozygous condition.

X. *up* (X-rays 3207r), used in females X/III.

XI. *mel* (X-rays, 2777r), presumably used in homozygous condition.

The table shows a number of very peculiar features.

(1) Well over 100,000 flies were scored in experiments in which the tester females were homozygous for a singly marked chromosome, and another 350,000 in experiments in which the tester females were heterozygous for such a chromosome. It is difficult to imagine why such an inefficient technique was chosen.

(2) No tester was used in both X-ray and AAM experiments; thus no locus was scored in the same genetical background after treatment with both agents.

(3) While seven alpha loci were tested with X-rays, only one of them was tested with AAM. It is difficult to imagine why the other six loci were tested at all.

(4) Only ten beta loci were tested with both mutagens. Again, it is difficult to imagine why the other six were tested at all. Unfortunately, the ten loci that were tested with both mutagens include not only bronzy which cannot be used, but also small-wing and notchoid which, by the original definition, are not beta loci (see under "Material").

(5) Throughout, smaller samples were used in the X-ray tests than in the chemical tests, although *ex hypothesi* the latter were expected to produce more mutants.

(6) Within one and the same test, the numbers scored often varied between chromosomes and even between loci on the same chromosome. This is the most peculiar feature of this peculiar table; it may throw some light on the method of scoring. The following discrepancies are found: *X-rays, 2990r:* 38,000 flies scored for the chromosome with the alpha loci, and 44,000 for the chromosome with the beta loci. This, as said before, may mean that two separate tests were done with the same dose. *X-rays, 4266r:* 35,000 chromosomes scored for *nd*, 22,000 for *smd*, both on the same chromosome. An explanation that comes to mind is that scoring of *smd* was discontinued after several mutations had been obtained at this beta locus.

X-rays, 3418r: 47,000 flies for the chromosome with the beta loci, 67,000 flies for the alpha locus *car* on the opposite chromosome, presumably, tests for *car* were continued until some mutations had turned up.

AAM, 2563"r": Here, every one of the five loci was tested on a different scale. For the beta loci, all on the same chromosome, the numbers counted ranged from 47,000 for *bz* to 87,000 for *ccw*. This suggests that scoring for any one beta locus was continued until it had mutated at least once. With *ccw*, this may have happened so late in the test that almost twice as many flies had to be scored as for *bz*. By far the largest numbers of flies were scored for *v*, on the opposite chromosome, an alpha locus expected to respond equally well to chemical treatment as to radiation.

Taken together, these data are strong presumptive evidence for the assumption that the number of flies scored depended on the locus to be tested. For most loci tested with X-rays, numbers from 30,000 to 40,000 were considered adequate and were adhered to. For beta loci in experiments with AAM, and for a few alpha loci in either series scoring appears to have been continued until the appearance of the first mutant or mutants. If this is the correct explanation of the remarkable data, it raises a difficult statistical problem; for it means that a comparison has to be carried out between experiments in which frequencies were determined by two different procedures. Obviously, the FAHMYS have disregarded this difficulty in their statistical treatment. Since, as will be shown, their findings are in any case without statistical significance, this additional source of error may be dis-

regarded; but it will have to be avoided or corrected for in any future tests of the same nature.

RESULTS

Tests of heterogeneity in Tables 1 and 2: In the FAHMYS' paper, Table 1 gives the numbers of mutations observed at 43 so-called alpha loci in response to treatment with X-rays and seven specified chemical agencies. The total of 549 mutations is distributed over the 344 cells, an average of about 1.6 occurrences per cell. Their Table 2 lists the results at 63 so-called beta loci of treatment by X-rays and five chemical agencies. The total for the 378 cells is 275 mutations, averaging about 0.73 per cell. They claim that Table 1 shows no significant heterogeneity, and that this proves that "the over-all relative mutability at the 'classical' or alpha loci, therefore, does not vary with the mutagen". For their Table 2 they claim significant heterogeneity, concluding that "the relative response of the beta loci does differ under the various mutagens".

On the plea that cell expectations are very small, the FAHMYS do not use the standard χ^2 test of heterogeneity. Nevertheless, it is worthwhile to carry out such tests on these tables, since it is generally accepted that the effect of small expectations is to inflate the χ^2 values, so that while a significant result must be treated with caution, a verdict of nonsignificance can be confidently accepted. We have therefore computed the χ^2 values and the corresponding P values by use of the Wilson-Hilferty formula. The results are

	D.F.	χ^2	P
Table 1	294	317.58	0.174
Table 2	310	347.99	0.072

Allowing for the fact that the χ^2 values are probably inflated because of the small expectations, this effect being much the greater for Table 2, there seems to be no statistically demonstrable heterogeneity in either table.

The FAHMYS do not give χ^2 figures, but use a test which they claim "is far less disturbed by small numbers in the individual cells than χ^2." This is the Ψ test of SMITH (1951, 1952), which they say yielded a heterogeneity P of 0.41 for Table 1 and 0.02 for Table 2. They do not make it clear which of the several possible variants of the test they used, but we have confirmed that it yields P values of the magnitude they claim, subject to one all-important proviso. The author of the test does *not* state that it is undisturbed by small expectations. On the contrary, he gives a suggested method for correcting for continuity, remarking that it probably over-corrects. When this correction is applied to Table 2, the P for heterogeneity changes from 0.02 to 0.99999999974, which means in effect that the Ψ test with SMITH's suggested correction breaks down. This is not surprising, since the correction consists in ignoring altogether deviations from expectation which are less than 0.5, and subtracting 0.5 from all other deviations; but 81 percent of the expectations in Table 2 are less than unity, and 56 percent are less than one half. The only reasonable conclusion is that heterogeneity in Table 2 has not been demonstrated.

Two very important points of principle arise. The FAHMYS explicitly claim that the inability of the test to demonstrate significant heterogeneity in Table 1 is a proof that the null hypothesis is true. This of course is completely unjustified. Of their 43 loci in Table 1, 19 mutated less than eight times, and seven mutated only twice. It would be a powerful statistical test indeed that could prove that these loci would react with equal proportionate frequency to eight different mutagenic agencies. In general, the null hypothesis of complete identity of behaviour can never be established. All that one can say, on adequately numerous observations, is that heterogeneity, if it exists, is unlikely to exceed certain confidence bounds. On the data of Table 1, these bounds are so wide as to be biologically meaningless.

Even if significant heterogeneity had been found in Table 2, this could not be taken as proof of differential susceptibility to mutagens until all other possible sources of heterogeneity had been examined and eliminated. Heterogeneity would arise from any departure in the experimental conditions from the strictest uniformity. In work with chemical mutagens, as in all pharmacological trials, it is quite common to find that experiments replicated as closely as possible will give heterogeneous results; and it is only when differences between treatments are significantly greater than those between replicates that an authentic effect can be inferred. It is not legitimate to rely on the inner conviction that one's technique is absolutely perfect. Even if further more extensive data should provide clear evidence of heterogeneity at different loci, this could not be accepted as evidence of differential specificity to mutagens until the requisite internal checks have been applied.

Tests of heterogeneity on Table 3: Table 3 of the FAHMYS is a 2×6 contingency table giving the number of mutations observed at the alpha and the beta loci under the action of X-rays and five different chemical mutagens. A χ^2 test for heterogeneity gives 18.8 for 5 degrees of freedom, which is highly significant. There is however a serious source of bias in their figures. The alpha loci were already known, and mutations produced by any agent, whether X-rays or chemicals, were presumably scored impartially as they occurred. But the beta loci, by definition, were those first identified by the FAHMYS as mutating under a chemical treatment. Every beta locus is therefore loaded with at least one mutation of chemical origin. Any mutation due to X-rays that occurred before the chemical mutation was observed would either not have been noticed, or would have disqualified the locus from inclusion in the beta category. There will therefore be a spurious excess of chemically induced mutations in the beta group as compared with the alpha group. The effect of this can be seen if Table 3 is summarized as a 2×2 table. This would read

	alpha loci	beta loci	
X-rays	57	19	76
Chemicals	403	256	659
	460	212	735

This gives a heterogeneity x^2 of 5.58, significant beyond the 2.5 percent level. But the beta loci-chemicals figure of 256 is artificially inflated. For a true correction, one would need access to all the experimental records. But a fairly good adjustment can be made by using the method of correcting for "rare ascertainment" in human pedigree studies, namely by deducting the event which brought the locus into the record, using subsequent mutations as an unbiassed sample of its behaviour. One therefore has to diminish the figure 256 by the 63 identifying chemically induced mutations. The revised table now reads

	alpha loci	beta loci	
X-rays	57	19	76
Chemicals	403	193	596
	460	212	672

This gives a heterogeneity of 1.70, which is clearly not significant.

When the X-ray row is deleted from the FAHMYS' Table 3, there is still apparently significant heterogeneity between the alpha and the beta loci with respect to the five kinds of chemical treatment; and the FAHMYS give a separate test purporting to show significant heterogeneity in the 2×2 table comprising rows 2 and 3 of their table, comparing the amino-acid mustards and the sulphonates. But there are 63 occurrences to be subtracted from the beta loci column, and until one knows by how much each of the cell entries need to be adjusted, it is not possible to draw any conclusions from the figures. It therefore would appear that no significant heterogeneity has been demonstrated between the totals for the alpha and the beta loci.

Specific loci tests. The Poisson distribution (Tables 4, 5, 6, 7) of the FAHMYS' *paper:* It would be tedious to explore all the misconceptions embedded in the short section on the Poisson distribution and the tests in Table 6 of the paper. Mention of two outstanding ones will suffice. The FAHMYS do not make clear what question they think that their tests are answering. Actually, their tests, if they have any validity at all, are testing the hypothesis that the *absolute* mutation rates at the various loci are identical. Of course, the disparate row totals in Table 1 and Table 2, as well as the whole mass of genetical experience, prove that they are not. Yet the FAHMYS seem pleased that their tests on seven loci, based on a total of 31 deficiencies and 21 visible mutations, appear to indicate that there is no significant departure from Poisson expectations in each of the two categories. The only conclusion that could be drawn, at best, is that their data are too sparse to reveal even the most elementary and best authenticated differences between loci.

There is however no warrant for drawing even this conclusion. The Poisson distribution deals with discrete events, and can be applied only to whole numbers. But the FAHMYS do not analyze whole numbers. They take the mutation rates per 10^8 chromosome r, rounded off to the nearest integer, and compare the variance of the rates with their mean. In the first row of their Table 6, for example, the mean mutation rate is 3.29 and its variance is 4.24, not significantly greater than the mean. But the use of 10^8 chromosome r is purely arbitrary. If they had

taken the rate per 10^9 chromosome r, for example, the mean would have been 32.9, and the variance 424.0, indicating dispersion tremendously greater than the Poisson expectation. Actually the χ^2 tests already considered are the most appropriate tests of conformation to Poisson expectation.

To these fallacies of the statistical treatment has to be added a further one, discussed above: the neglect of the statistical problem how to deal with frequencies that have been obtained in different ways for different series.

It remains to discuss the claim that certain loci are "refractory to X-rays," to the extent that the chromosome regions including these loci cannot be broken by X-rays. The eight "refractory" loci are *brc*, *nd*, *sma*, *cop*, *tc*, *sl*, *Tu*, *mel*. They will be considered in turn.

brc lies at 0.0, i.e. in the same region as scute. Two scute deficiencies were produced in the X-ray experiments; many more have been produced by other workers. It is impossible to claim that this region is refractory to X-ray breakage, unless one postulates that its response to radiation in spermatozoa depends on whether the ovum subsequently to be fertilized carries an alpha or beta locus at 0.0. Since homozygotes for *brc* are reported to be viable, zygotic inviability cannot be invoked as explanation.

nd is an allele of Notch and can be uncovered by Notch deficiencies. Twelve such deficiencies were, indeed, obtained in the experiments with AAM. Surprisingly, none occurred among 35,000 irradiated chromosomes. Even so, the fact that this region is easily broken by irradiation is too well established to be overthrown by this one negative result.

cop 67,000 chemically treated chromosomes contained two deficiencies (or lethal mutations) for this locus; 29,000 irradiated chromosomes contained none. This is hardly evidence for a specifically refractory attitude towards radiation.

tc No deficiency in 49,000 chemically treated and 44,000 irradiated chromosomes.

sl No deficiency in 74,000 chemically treated and 44,000 irradiated chromosomes.

Neither of these two loci can, therefore, be said to be specifically refractory to X-rays.

The last three "refractory" loci, *sma*, *Tu*, and *mel*, were tested only with X-rays and are therefore useless for comparion with AAM.

It is evident that there is no reason for believing that any one of these eight loci possesses the peculiar ability of endowing its chromosomal environment with resistance to X-ray breakage.

DISCUSSION

Geneticists must welcome the paper by FAHMY and FAHMY (1959a), which for the first time presents data on the specific effects of chemical mutagens in Drosophila, so long proclaimed by its authors. Unfortunately, it does not bring sufficient data, nor does it describe details of experimental procedure that are indispensable for critical appraisal of the evidence. Faults of both omission and

commission in the presentation suggest that the procedure was inadequate in many respects.

The setting-up of two mutually exclusive classes of loci, differing in mutability under the influence of various mutagens, is gratuitous, unsupported by evidence, and an obstacle to further research. The FAHMYS, in the discussion of their paper, admit that their present classification of gene loci is "neither rigid nor final," but they claim to have "clearly shown the reality of such a classification." Our examination of their data has shown this claim to be unjustified. Their criteria of classification are manifold and have been used inconsistently, the only indispensable criterion of beta mutants being discovery by the FAHMYS. In particular, we have shown that the FAHMYS have failed to establish the two alleged main criteria of beta loci: low mutational response to radiation and marked differential response to various mutagens. The statistical tests purporting to establish these beta properties have been shown to be faulty. Even if this had not been so, the data for statistical treatment had been collected in such a way that in no case could the results have been interpreted in terms of differential mutagen sensitivity at the tested loci.

This critique must not be taken as implying that there is no differential susceptibility to mutagens at different loci of Drosophila. On general biological grounds, including the references given by the FAHMYS in their introductory section, one would assume that such differences may exist. The data presented by the FAHMYS do not disprove this possibility, even though they fail to support it. Clear evidence for mutagen specificity in Drosophila will not be easy to obtain because of the necessarily very limited scale of the experiments. A hypothesis regarding the causes of possible specificity might reduce the required scale. Thus, the fairly well established fact (FAHMY and FAHMY 1956; BELITZ 1957; FAHMY and FAHMY 1959a) that certain chemical mutagens tend to produce mutations in the proximal region of the X chromosome might be taken as a possible starting point. If, as this observation suggests, there is a regional specificity of mutagenic response, autosomal mutations, too, might tend to occur in heterochromatic or else centromere-near regions after treatment with triethylenemelamine or amino-acid mustards. The influence of the centromere might be studied by following the suggestion, made several years ago by DR. MULLER, that an inverted X chromosome should be used for treatment. Should regional specificity become sufficiently confirmed, more effective specific loci tests than those used so far might be designed by choosing loci within the preferred regions. Perhaps it is not accidental that in the very large-scale tests on the *car* locus no *bona fide* mutation occurred after irradiation, although two had occurred in a, presumably, very much smaller sample of chemically treated chromosomes used for Muller-5 tests.

Regional specificity is, in any case, the more likely explanation for those cases in which a locus as a whole seems to respond better to one mutagen than another; for Drosophila loci, like those of microorganisms, are presumably complex and contain subloci with different mutational potentialities. From this point of view, the occurrence of unusual alleles at known loci, such as notchoid or scute^{fahmy} is of special interest. One way of testing specificity at this level would be to de-

termine induced frequencies of reverse mutations in a series of pseudoalleles. A different method, which appears to have been taken up by the FAHMYS (1959b), consists of dividing all induced mutants at one locus into groups by tests of recombination and complementation, in the hope of finding group differences in mutagen specificity.

We should be glad if our critique would clear the way for a really rewarding approach to the interesting problem of mutagen specificity in Drosophila. The more plausible and congenial a hypothesis, the more necessary it is to be content with nothing less than water-tight reasoning from adequate and unbiassed observation.

SUMMARY

This paper presents a detailed examination of the claim (FAHMY and FAHMY 1959a) that the Drosophila genome consists of two classes of loci, differing in mutational capabilities. It has been concluded that the biological techniques used were inadequate for an unbiassed test of this hypothesis, and that the statistical techniques contained various fallacies and did not, in fact, prove what they were supposed to have proved. It is stressed that this conclusion does not exclude the possibility of mutagen specificity in Drosophila, but that different experiments will be required for proving it.

LITERATURE CITED

AUERBACH, C., 1954 Sensitivity of the Drosophila testis to the mutagenic action of X-rays. Z. Ind. Abst. Vererb. **86**: 113–125.

BELITZ, H. J., 1957 Vergleichende Untersuchungen der Verteilung spontaner und durch Chinon I (Bayer 4073) induzierter Mutationen über die genetische Karte des X-Chromosoms von *Drosophila melanogaster*. Z. Ind. Abst. Vererb. **88**: 434–442.

BRIDGES, C. B., and K. S. BREHME, 1944 *The Mutants of Drosophila melanogaster*. Carnegie Inst. Wash. Publ. **552.**

FAHMY, O. G., and M. J. FAHMY, 1955 Cytogenetic analysis of the action of carcinogens and tumour inhibitors in *Drosophila melanogaster*. IV. The cell stage during spermatogenesis and the induction of intra- and intergenic mutations by 2:4:6-tri(ethyleneimino)-1:3:5-triazine. J. Genet. **53**: 563–584.

1956 Cytogenetic analysis of the action of carcinogens and tumour inhibitors in *Drosophila melanogaster*. V. Differential genetic response to the alkylating mutagens and X-radiation. J. Genet. **54**: 146–164.

1958a New mutants of *Drosophila melanogaster*. Drosophila Inform. Serv. **32**: 67–78.

1958b Discussion: Mutagenic effects of alkylating agents. Ann. New York Acad. Sci. **68**: 736–748.

1959a Differential gene response to mutagens in *Drosophila melanogaster*. Genetics **44**: 1149–1171.

1959b Complementation among the subgenic mutants in the *r*-locus of *Drosophila melanogaster*. Nature **184**: 1927–1930.

GLASSMAN, E., 1959 Allelism and complementation of bronzy *(bz)* and maroon-like *(ma-l)* eye color mutants in *D. melanogaster*. Drosophila Inform. Serv. **33**: 135.

REDDI, O. S., 1960 Sensitivity of the Drosophila testis to tri-ethylene melamine (TEM). Genet. Res. (In press).

SMITH, C. A. B., 1951 A test for heterogeneity of proportions. Ann. Eug. Lond. **16**: 16–25.

1952 A simplified heterogeneity test. Ann. Eug. Lond. **17**: 35–36.

TRANSLOCATIONS BETWEEN THE X AND THE Y CHROMOSOMES OF DROSOPHILA MELANOGASTER

BENEDETTO NICOLETTI[1] AND DAN L. LINDSLEY

Biology Division, Oak Ridge National Laboratory,[2] Oak Ridge, Tenn.

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INDUCED chromosomal rearrangements in Drosophila have generally been recovered from cells that were in postmeiotic stages, i.e., spermatids and spermatozoa, at the time of treatment. Since the X and the Y chromosomes segregate into separate cells at the first meiotic division, recovery of interchanges between these two elements has therefore been precluded. Induction of X;Y translocations (i.e., T(X;Y)'s) in mature sperm can be accomplished by treating males that carry an extra Y chromosome. These males produce sperm carrying both the X and the Y in one third or more of the cases.

PANSHIN (1941a,b) has recorded recovery of seven reciprocal translocations between the X and the Y from irradiated *In(1)w*^{m4}/Y/Y males. He reports that the translocations were recognized because T(*In(1)w*^{m4};Y)/*y w f bb* females show less severe variegation than *In(1)w*^{m4}/*y w f bb*/Y females. He selected slightly variegated females and recorded as T(X;Y)'s those that produced a viable aneuploid class of progeny. Since these translocations were incidental to the purpose of PANSHIN's experiments, however, he did not study them further.

Translocations between compound X chromosomes and the Y have been induced by irradiation of oocytes and extensively studied by several investigators (PARKER 1953, 1954; MULLER and HERSKOWITZ 1954; ABRAHAMSON, HERSKOWITZ, and MULLER 1956; PARKER and MCCRONE 1958; PARKER and HAMMOND 1958). This method, however, leads to the recovery of only half of each induced translocation as a detachment; the other half is included in a polar nucleus during meiosis and therefore lost. Furthermore, detachments are a special sample of X;Y translocations, since the distribution of break points in the X is restricted by the limited degree of aneuploidy that is compatible with recovery of a detachment. It is therefore clear that the oocyte method is not suitable for an exhaustive study of interchange between the X and the Y chromosomes.

Rearrangements involving the X chromosome are likely to be associated with recessive lethality (PATTERSON, STONE, BEDICHEK, and SUCHE 1934) or male sterility (SCHULTZ 1947; LINDSLEY, EDINGTON, and VON HALLE 1960); rearrangements involving the Y chromosome are likely to be associated with male sterility (NEUHAUS 1939). Consequently, to get an unbiased estimate of involvement of either X or Y in induced rearrangements, the irradiated genome should be recovered in F_1 females rather than males, as is commonly done. This pre-

[1] Present address: Istituto di Genetica, Università di Roma, Rome, Italy.

[2] Operated by Union Carbide Corporation for the U.S. Atomic Energy Commission.

caution was taken in the present experiments, and subsequent analysis of the translocations recovered showed that the yield of T(X;Y)'s was thereby approximately doubled.

Because a representative array of interchanges between the X and the Y had never been studied and because the special properties of these chromosomes (STERN 1927; COOPER 1959) made it seem likely that interchanges between them would have interesting properties, we undertook a study of T(X;Y)'s. A sample of 86 T(X;Y)'s was produced by irradiation of X/Y/Y males, and the genetic properties as well as the polytene cytology of each were investigated. The viability and fertility of each translocation both as T(X;Y)/Y and T(X;Y)/0 males were determined; the salivary chromosome break point in the X and the arm of the Y involved were also determined, and the viability of the various types of aneuploid progeny produced by each translocation was recorded.

Owing to the relative unimportance of Y chromosome aneuploidy to the viability of the individual, T(X;Y)'s provide particularly useful cytogenetic tools for manipulating X chromosome constitution. For example, the possibility of using T(X;Y)'s to construct Y chromosomes marked with almost any region from the X has already been demonstrated by the synthesis of Y chromosomes carrying the normal alleles of *w* and *N* on the short arm, i.e., Yw^+ (BROSSEAU, NICOLETTI, GRELL and LINDSLEY 1961).

Recovery of T(X;Y)'s

The crossing scheme used for the recovery of translocations between the X and the Y is outlined in Figure 1. X/Y/Y males that were $y/y^+Y/B^sY$ in con-

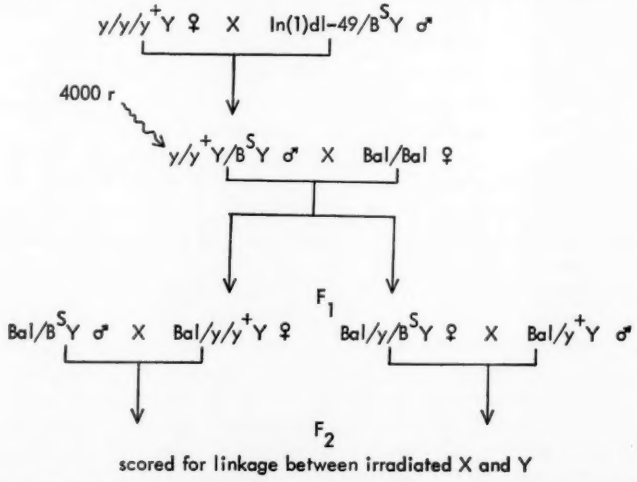
y/y/y+Y ♀ X In(1)dl-49/BSY ♂
4000 r
y/y+Y/BSY ♂ X Bal/Bal ♀
F1
Bal/BSY ♂ X Bal/y/y+Y ♀
Bal/y/BSY ♀ X Bal/y+Y ♂
F2
scored for linkage between irradiated X and Y

FIGURE 1.—The crossing scheme used in the production and recovery of translocations between the X and the Y chromosomes. Bal refers to the Balancer chromosome, which was $Y^sX{\cdot}Y^L$, *Ins(1)EN*, dl-49, *KS y v f car · KL* in some cases and *Ins(1)sc*^{s}, dl-49, y^{sid} v^{of} *f* in others.

stitution were irradiated with 4000r (250 kvp, 30 ma, 3 mm of Al filter) and, immediately after exposure, were mass-mated with females homozygous for a balancer chromosome (Bal ♀♀), and a five-day egg sample was collected. The y^+Y chromosome (*sc*^{s}·Y of MULLER 1948) carries the normal allele of yellow, y^+, on the tip of the long arm, and the B^SY (BROSSEAU *et al.*, 1961) is marked on Y^L with the B^S derived from T(1;4)B^S. Virgin females of the constitution Bal/y/Y were recovered from the cross of the irradiated males with the Bal females and mated individually to males carrying the same balancer chromosome. These males carried the alternative marked Y chromosome, to allow both Y's to be followed in subsequent crosses. The X;Y translocations were recognized by the occurrence of linkage between the maternally derived X and Y chromosomes in the progeny of the pair matings. As previously mentioned, rearrangements between the Y and the autosomes are frequently male sterile, and rearrangements between the X and the autosomes are frequently male lethal or sterile. Because we anticipated a compounding of these behaviors for the T(X;Y)'s, the crosses were designed in such a way as to recover these rearrangements in daughters of the irradiated males (Bal/T(X;Y) ♀♀).

The balancer chromosome used in this scheme was Y^SX·Y^L, *Ins(1)EN*,dl-49, *KS y v f car-KL*, where *KS* and *KL* refer to the fertility complexes of Y^S and Y^L respectively (BROSSEAU 1960) in some cases and *Ins(1)sc*^{s},dl-49, y^{s1d} v^{of} *f* in others. The presence of recessive markers *f* and *car* provided additional aid in recognizing aneuploid types among the progeny of Bal/T(X;Y) females.

The translocation process breaks the X into an acentric distal portion (X^D) and a centric proximal portion (X^P). The distal portion is appended to the centric proximal portion of the Y(Y^P), and X^P is capped by the distal acentric portion of the Y(Y^D). When the Y break is in the marked arm, X^P carries the Y derived marker (Figure 2a); and when the Y break is in the unmarked arm, X^D carries the marker (Figure 2b).

Forgetting for a moment the Y constitution, a T(X;Y)/Bal female may be designated X^D/X^P/B and the products of meiosis as X^D/X^P and B; X^D and X^P/B; X^P and X^D/B; X^D/X^P/B and 0. Fertilization of ova of these constitutions by B- or Y-bearing sperm produces the various types of aneuploid zygotes enumerated in Figure 3. The number of surviving aneuploid types varies according to the position of the break point in X. The ranges of break points (column 1 of Figure 3) that produce the various combinations of surviving aneuploid types enumerated in Figure 3 have been estimated mostly from the surviving aneuploids observed in the current studies. The recognition of a T(X;Y) depends on the absence of aneuploid classes from the progeny of the F_1 female; consequently, where several of the aneuploid classes survive, ambiguities in classification may arise. Examination of the first line in Figure 3 shows that, if the break point in X is sufficiently distal [i.e., to the left of *l(1)J1* except in some combinations with y^+Y], all the aneuploid types survive. If the break in Y is in the short arm so that X^D becomes marked (i.e., associated with the Y chromosome derived marker), X^D and X^P are indistinguishable from the marked Y and the normal X, respectively, and the progeny will not be recognized as deriving from a transloca-

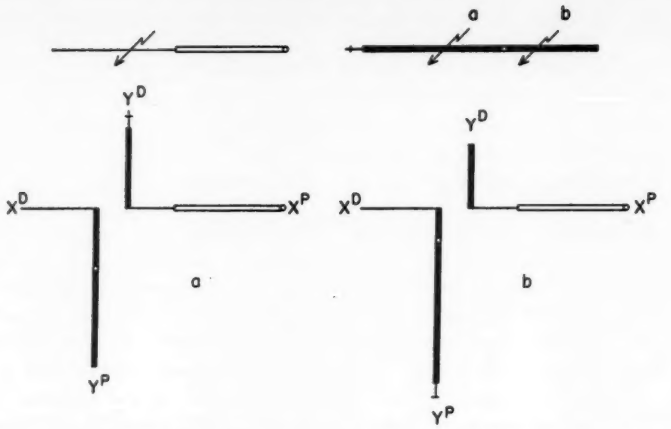

FIGURE 2.—Formation of translocations between the X and Y^L (a) or Y^S (b) with the structure and marker content of the resulting elements.

tion heterozygote. If, on the other hand, the Y is broken in the long arm and X^P becomes marked, then although X^D is a small element, the Y-derived marker and the X segregate together, and the presence of a T(X;Y) is recognized. If the break in X is slightly more proximal (line 2 of Figure 3), males carrying X^P but not X^D will succumb but the other aneuploid types will survive. Depending on the position of the Y break point as outlined, this type of T(X;Y) may be scored unambiguously as a translocation, or it may be misclassified as a sex-linked recessive lethal unassociated with the Y chromosome. Even this ambiguity disappears for breaks proximal to band 1C3, owing to the extreme Minute phenotype of X^P/X females (cf., *Df(1)Bld* = *M(1)Bld*). It is evident that ambiguities in classification of T(X;Y)'s with distal X chromosome break points are indeed quite trivial.

Consider now T(X;Y)'s in which the X is broken proximally. As indicated in line 7 of Figure 3, when the X is broken in the chromocentral heterochromatin, all four aneuploid classes survive. The survival of X^D in the absence of X^P is attributable to the fact that the resulting deficiency involves the region of X-Y homology and is covered by the paternally derived Y. When the Y break is in the short arm, linkage is established between the Y marker and the X, and a translocation is recognized, although X^P, which is capped by the unmarked tip of the Y, is unrecognizable and likely to be lost. When Y is broken in the long arm, however, X^P is marked and is indistinguishable from a nontranslocated Y, and X^D is unmarked and resembles a nontranslocated X. Because a high proportion of X chromosome rearrangements are known to have breaks in the proximal heterochromatin (BAUER, DEMEREC, and KAUFMANN 1938), this ambiguity is expected to result in the failure to detect an appreciable fraction of the translocations, i.e., those between the proximal heterochromatin of the X and the long

arm of the Y, i.e., T(Xh;Y^L)'s (Xh = the chromocentral heterochromatin of the X; after Cooper 1959). Breaks in X immediately distal to the region of homology between X and Y can lead to a T(X;Y), in which X^D/Y is inviable but X^D/X survives (line 6, Figure 3). Such a translocation is scorable if X^D is marked, but it will resemble a sex-linked lethal that does not involve the Y if X^P is marked. As in the case of distal breaks in X, this ambiguity is made less serious by the Minute phenotype of X^D/X when the X break is distal to the locus of *M(1)n*, which, although not localized on the salivary map, is proximal to *car* at 62.7 on the genetic map. The use of a Y chromosome marked terminally on both arms would have circumvented all the ambiguous cases described by rendering both X^P and X^D marked in every T(X;Y); furthermore all aneuploid individuals

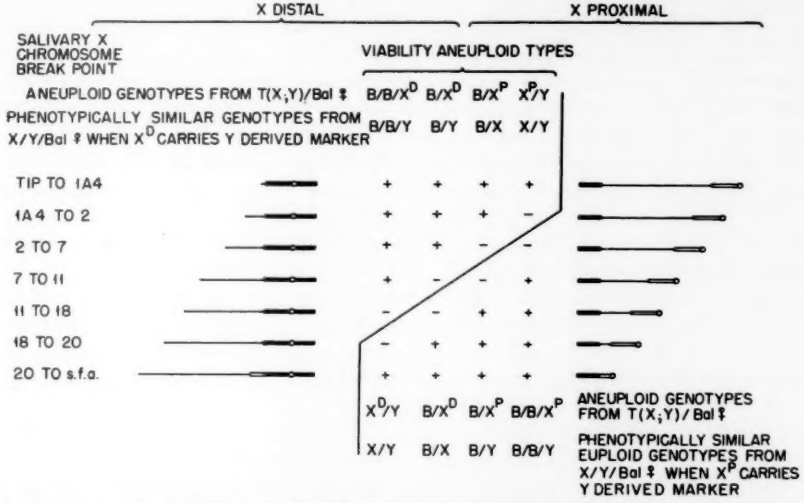
X DISTAL
X PROXIMAL
SALIVARY X CHROMOSOME BREAK POINT
VIABILITY ANEUPLOID TYPES
ANEUPLOID GENOTYPES FROM T(X;Y)/Bal ♀
B/B/X^D B/X^D B/X^P X^P/Y
PHENOTYPICALLY SIMILAR GENOTYPES FROM X/Y/Bal ♀ WHEN X^D CARRIES Y DERIVED MARKER
B/B/Y B/Y B/X X/Y
TIP TO 1A4
1A4 TO 2
2 TO 7
7 TO 11
11 TO 18
18 TO 20
20 TO s.f.a.
X^D/Y B/X^D B/X^P B/B/X^P
ANEUPLOID GENOTYPES FROM T(X;Y)/Bal ♀
X/Y B/X B/Y B/B/Y
PHENOTYPICALLY SIMILAR EUPLOID GENOTYPES FROM X/Y/Bal ♀ WHEN X^P CARRIES Y DERIVED MARKER

Figure 3.—The viability of the various aneuploid genotypes resulting from the cross of T(X;Y)/Bal ♀ × Bal/Y ♂ as a function of the break point in the translocated X chromosome. Also indicated are the euploid genotypes produced among the progeny of X/Y/Bal ♀ × Bal/Y ♂ that are phenotypically similar to each aneuploid type and with which the aneuploid type can be confused leading to failure to recognize translocation bearing cultures. The aneuploid genotypes to the left of the diagonal may be confused with the corresponding euploid genotype only if the portion of the translocation to the left of the diagonal (i.e., X^DY^P) carries the Y-derived marker, and the aneuploid genotypes to the right of the diagonal may be confused with the corresponding euploid genotype only if the portion of the translocation to the right of the diagonal (i.e., Y^DX^P) carries the Y-derived marker. The ranges of break points giving rise to translocations with similar aneuploid survival patterns have been derived mostly from observations on the translocations investigated in the present work; they are only rough estimates and are intended as an approximate illustration rather than an accurate indication of the points at which the characteristics of T(X;Y)'s change. The narrow line represents X euchromatin; the open heavy line, X heterochromatin; and the solid heavy line, the Y chromosome. X^DY^P has been abbreviated X^D, Y^DX^P has been abbreviated X^P and the Balancer chromosome has been abbreviated B. The aneuploid class X^P/B includes X^P/B′ and X^P/B/Y′, where the primed element is of paternal origin; similarly X^D/B = X^D/B′ + X^D/B/Y′; B/Y = B′/Y + B/Y/Y′; and B/X = B′/X + + B/X/Y′.

would be recognizable by the fact that they carry but one of the Y chromosome-derived markers. Unfortunately, however, the $B^sY\gamma^+$ (BROSSEAU 1958) was not available at the outset of these experiments.

Four experiments were carried out to induce T(X;Y)'s, and the results are presented in Table 1. The incidence of translocations in the fourth experiment seems to be higher than that observed in the other three. In this experiment the Bal/X/Y females carried the balancer *Ins(1)sc*^{s},dl-49, y^{31d} v^{0f} *f* rather than $Y^sX{\cdot}Y^L$, *Ins(1)EN*,dl-49, *KS y v f car-KL* used in the other experiments. There is no obvious way, however, that this change in procedure could account for the observed difference. It is also apparent from Table 1 that many more B^sY-bearing sperm than γ^+Y-bearing sperm were sampled; yet from Figure 1 it can be seen that equal numbers should have been produced. The explanation for this is that the $Y^sX{\cdot}Y^L/B^sY$ males to which Bal/X/γ^+Y females were crossed were quite infertile, and consequently many of these pair matings failed. γ^+Y-bearing sperm could not be scored in experiment three because linkage between γ^+Y and the X arose spontaneously in the stock, presumably through an exchange between Y^s and Xh. It can be seen that translocations involving B^sY and γ^+Y are recovered with equal frequency; consequently, the marker difference does not visibly affect their breakage properties. Correcting the total incidence of translocations upward in an attempt to include the previously discussed types that could not be scored (see also section on cytological analysis), we arrive at a value of approximately two percent T(X;Y)'s produced at 4000r. On the basis of relative metaphase lengths of the X, the Y, and the autosomes, this figure is in reasonable agreement with the 7–9 percent T(2;3)'s induced in sperm by 4000r (MULLER 1940; CATSCH 1948). In both MULLER's and CATSCH's experiments and in the current work heterogeneous sperm samples were used, in that irradiated males were left with females for a number of days.

TABLE 1

Data on recovery of T(X;Y)'s by the method outlined in Figure 1

Experiment	Y	Gametes tested	T(X;Y)'s recovered	
			Number	Percent
I	γ^+Y	277	1	0.36
	B^sY	391	4	1.02
II	γ^+Y	296	2	0.67
	B^sY	586	3	0.51
III	B^sY	1673	18	1.08
IV	γ^+Y	1078	19	1.76
	B^sY	2247	40	1.78
Total	γ^+Y	1651	22	1.33
	B^sY	4897	65	1.33
Grand total		6548	87	1.33

The T(X;Y)'s were temporarily carried with the Balancer used for their recovery, and data were accumulated on the survival and phenotype of the various aneuploid types. This Balancer, which was homozygous viable and fertile, was subsequently replaced by a derivative of FM4 kindly provided by DR. E. H. GRELL. This chromosome, although having the same sequence as FM4 [*Ins(1)sc*^{s}, dl-49,3C-4EF; see MISLOVE and LEWIS 1954], is marked with y^{31d}, *w*, *dm*, and *f*; it combines the advantage of the female sterility of *dm* and the larval marker, *w*. Male fertile T(X;Y)'s were also kept in stocks with a reversed acrocentric X (MULLER's double X). The transmissibility of the different T(X;Y)'s is variable, but it has not been determined whether this is caused by meiotic phenomena or by reduced viability of translocation-bearing progeny.

Male viability and fertility

The scheme for recovering the T(X;Y)'s was designed to permit detection of the translocation in a progeny containing both T(X;Y)/Bal females and T(X;Y)/Y males. The survival of translocations with an associated sex-linked recessive lethal was assured by the presence of the heterozygous females, and the free Y in the translocation-bearing males was provided to overcome any impairment of fertility caused by the break in Y. The 87 translocations recovered are classified according to the Y and the Y arm involved as well as according to viability and fertility of T(X;Y)/Y males (Table 2). The determination of the Y arm involved in the translocation was based on the distribution of the Y-derived marker among the aneuploid types in combination with the cytological analysis. One fourth of the T(X;Y)'s (22/87) fail to survive as males; some of these sex-linked recessive lethals may have been induced independently of the T(X;Y), but the majority are almost certainly associated with the X chromosome break point of the rearrangement. All translocations that survive as T(X;Y)/Y males were tested as T(X;Y)/0 males; two were inviable in this combination. Of the 65 male viable translocations, 22 were sterile as T(X;Y)/Y males. This sterility cannot be attributed to the inactivation or loss of fertility factors from the translocated Y, since it is unaffected by the presence of the extra normal Y. We

TABLE 2

Genetic classification of the T(X;Y)'s studied correlated with cytological information regarding the arm of the Y involved

Y chromosome	Arm involved	♂ Fertile	♂ Sterile	♂ Lethal	Total
y^{+}Y	Long	4	6	3	13
	Short	5	1	3	9
	Total	9	7	6	22
B^{s}Y	Long	19	8	8	35
	Short	15	7	8	30
	Total	34	15	16	65
	Grand total	43	22	22	87

postulate that it is associated with the rearrangement of the X since a high correlation between male sterility and rearrangements involving the X chromosome has been observed by other investigators (SCHULTZ 1947; and LINDSLEY *et al.* 1960). Forty-three translocations were T(X;Y)/Y viable and fertile; they were crossed to XX/0 females and their T(X;Y)/0 sons were checked for viability and fertility. All 43 were viable in the absence of the extra normal Y, but only 14 were fertile. In fertile T(X;Y)/0 males, we observed that the two halves of the translocation regularly pair with and separate from each other at the first meiotic division in such a way that every sperm produced carries one half of the translocation. Consequently, when such males are crossed to attached-X females, attached-X daughters hyperploid for one of the halves of the translocation are the only progeny produced. Crosses of T(X;Y)/0 males to T(X;Y)/X females or to the hyperploid attached-X females, when they were fertile, however, produced both sons and daughters owing to the ability of these females to produce gametes with an aneuploid constitution complementary to that produced by the male. The 29 translocations that were fertile as T(X;Y)/Y but sterile as T(X;Y)/0 represent instances of inactivation or loss of Y chromosome fertility factors since the sterility can be overcome by the presence of a normal Y.

Cytological analyses

The salivary gland chromosomes of each of the 87 translocations were examined and the break points determined. X;Y translocations generally assume one of two configurations in salivary preparations. The centric ends of both halves of the translocation (Figure 2) are associated with the chromocenter. The distal end of X^p, which is capped by Y^D, may be associated with the chromocenter to form a loop (Figure 4a,b), or it may be free of the chromocenter (Figure 4c,d,g). The frequency with which Y^D associates with the chromocenter is a function of the length of X^p, so that when X^p is short Y^D is likely to be in the chromocenter and when X^p is long Y^D is likely to be free. It has also been noted that, when X^D is short the tip of the X is likely to become associated with the chromocenter. Furthermore, capping of X^p with the B^S marker region predisposes X^p to loop formation, possibly owing to the tendency of the su^w-f region of B^SY to pair with its homologous region in the X heterochromatin. Where possible, male larvae were examined, since they generally exhibited the configurations just described. In the male lethal translocations, however, the translocations were studied in female larvae in combination with a normal sequence X, where pairing between X^D and the normal homolog tended to confine the break-point region to the chromocenter (Figure 4e). The observations were facilitated when X^D was broken away from the chromocenter (Figure 4f,h). In addition to simple reciprocal translocations between the X and the Y, a number of more complex rearrangements involving the autosomes as well as the X and Y were encountered. In most of what follows, however, only the breaks involved in X;Y interchange are considered.

These translocations should provide a unique opportunity for the examination of polytene morphology of the Y chromosome, which was reported by

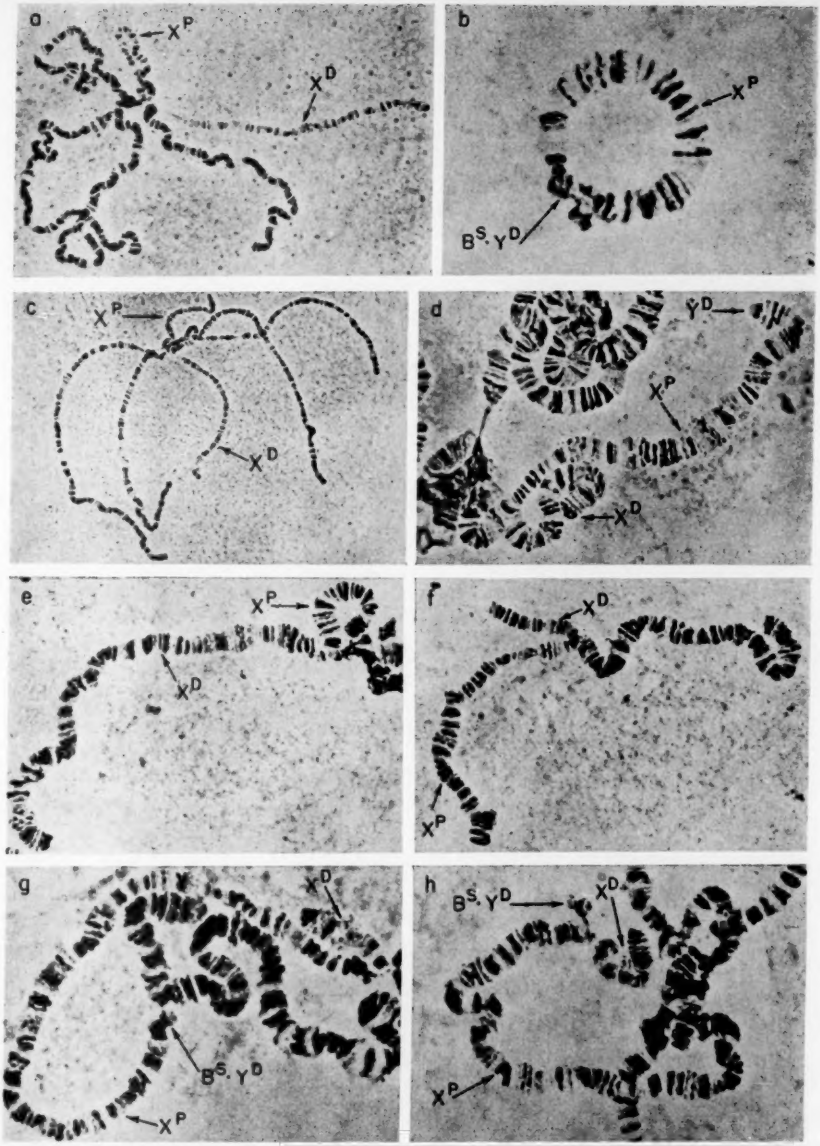

FIGURE 4.—Representative cytological configurations of X;Y translocations in males (a,b,c,d,g) and in heterozygous to a normal X females (e,f,h). a, T(X;Y^L) #130, X break point 11F; b, T(X;Y^L) #240, X break point 14A; c, T(X;Y^S) #169, X break point 11D; d, T(X;Y^S) #131 X break point 6E; e, T(X;Y^L) #142, X break point 13E; f, T(X;Y^L) #142; g, T(X;Y^L) #109, X break point 3C; h, T(X;Y^L) #114, X break point 3C.

PROKOFYEVA-BELGOVSKAYA (1937) to consist of 8 to 10 thin chromocentral bands. In no case, however, did we encounter any features that could clearly and consistently be identified with the Y chromosome (Figure 4d), although its euchromatic marker segment was commonly observed. The B^s marker segment is a discrete element consisting of three heavily staining bands (BROSSEAU *et al.*, 1961), and it was easily recognized either when associated with X^D (Figure 4g,h) or X^P. The y^+ marker, on the other hand, is recognizable only by virtue of its occasional pairing with the tip of the X. The position of the marker segment was useful in determining which arm of the Y was involved in the translocation. No attempts were made to localize the Y break point more accurately by analysis of metaphase chromosomes.

The X chromosome break points were localized within the limits of the lettered subdivisions of BRIDGES' revised salivary map (1938). For a complete tabulation of the salivary chromosome breakpoints and the genetic characteristics of each translocation see NICOLETTI and LINDSLEY (1960). The frequency of breaks encountered in each of the 20 major divisions is plotted for Y^L and Y^S of B^sY and y^+Y in Figure 5. Several features of this histogram are immediately apparent. First there seem to be regions preferentially involved in recoverable T(X;Y)'s that are inferentially regions of high breakability—regions 3, 7, and 11 for T(X;Y^L)'s and region 20 for T(X;Y^S)'s. The apparent high breakability of the three euchromatic regions, although based on a relatively small sample of breaks, is in agreement with KAUFMANN's finding (1946) that, among others, regions 3C, 7B, and 11A have high coefficients of breakage. It is not known whether the failure of rearrangements involving Y^S to exhibit similar preferential euchromatic breakability represents a real difference between Y^S and Y^L or is a sampling artifact. KAUFMANN has estimated that approximately 30 percent of the X chromosome breaks are in the proximal heterochromatin, i.e., regions 19F and 20. The present observations reveal that 50 percent of the T(X;Y)'s involving Y^S [i.e., T(X;Y^S)'s] have the X chromosome break in the proximal hetero-

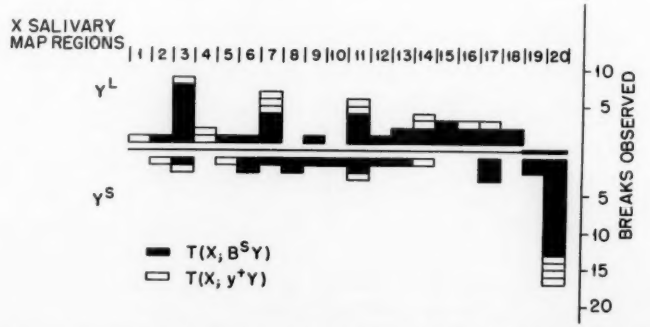

FIGURE 5.—Frequency distribution of the X chromosome break points from 86 T(X;Y)'s according to polytene chromosome region. The breaks in regions 19E and F have been pooled with those in region 20.

chromatin, but the corresponding class involving Y^L was not recovered. The reason that T(X;Y)'s with breaks in Y^L and Xh are not recovered has been thoroughly discussed in the section describing the method for recovery of translocations. Briefly, each half of such a translocation is able to survive in the absence of the other, and the linkage between the X markers and the Y markers is not established (Figure 3, line 7). Since breakage is equally frequent in the Xe and Xh among the T(X;Y^S)'s, we can estimate that about 45 T(X;Y^L)'s were produced but not recovered by assuming the same proportions of Xe to Xh breaks among T(X;Y^L)'s. These 45 postulated translocations were included in the computations of the total translocation frequencies presented earlier. It should be noted, however, that this figure could be an overestimate if KAUFMANN's estimate (1946) that 30 percent of X breaks are in Xh is closer to the correct value than our estimate of 50 percent. KAUFMANN's figure was very approximate because he had to estimate the contribution to Xh breakage of wholly heterochromatic rearrangements, which he was unable to detect. On the other hand, we believe that we have detected every T(X;Y^S) and therefore have a much more reliable estimate of the relative proportion of Xe and Xh breakage. The possibility exists, however, that the homology between Xh and Y^S may lead to spatial relations within the sperm head that produce preferential involvement of Y^S and Xh in T(X;Y)'s; in this case the ratio T(Xe;Y^S):T(Xh;Y^S) would not estimate the corresponding ratio for Y^L, i.e., T(Xe;Y^L):T(Xh;Y^L).

Correlation of the cytological and genetic observations

The cytological and genetic characteristics of 86 T(X;Y)'s are correlated in Table 3. The 87th translocation did not involve an X;Y interchange, as will be described.

The cytological information can be used in obtaining a more precise picture of the nature of male sterility. It will be recalled that there are two types of male sterility: one type is expressed in the presence of a normal Y chromosome and

TABLE 3

Correlation of the cytological and genetic analyses of 86 translocations between the X and the Y chromosomes

Genetic description*				Cytological classification				
T(X;Y)/Y		T(X;Y)/0		Xe		Xh		
Viable	Fertile	Viable	Fertile	Y^L	Y^S	Y^L	Y^S	Total
—	nt	nt	nt	11	9	0	2	22
+	—	—	nt	2	0	0	0	2
+	—	+	nt	11	7	0	1	19
+	+	—	nt	0	0	0	0	0
+	+	+	—	14	0	0	15	29
+	+	+	+	9	4	0	1	14
			Total	47	20	0	19	86

* —, Tested and found to be negative. nt=not tested or not testable.

has been attributed to the X chromosome, and the other type is expressed only in the absence of a normal Y and has been attributed to inactivation or loss of Y chromosome fertility factors. The sample of 21 T(X;Y)/Y sterile translocations contained 13 of the 17 cytologically detectable T(X;Y;A)'s; the four other T(X;Y;A)'s were found among the lethal translocations. It is conceivable that, were the lethality covered, these too would prove to be male sterile. This observation corroborates the correlation between X;A translocation and male sterility noted by SCHULTZ (1947) and more recently by LINDSLEY *et al.* (1960). It was considered likely that the nine male sterile translocations that were not observed cytologically to involve an autosome were actually T(X;Y;A)'s in which the autosomal break point was in the proximal heterochromatin. To check this supposition seven of these nine cases were tested genetically for linkage between the T(X;Y) and the two major autosomes; three of them proved to be T(X;Y;A)'s, and the other four could not be shown to carry an autosomal rearrangement. LINDSLEY *et al.* (1960) observed that T(X;A) associated male sterility is characterized by failure of sperm head elongation. An examination of the testes of three of the sterile T(X;Y;A)'s revealed the same phenotype of sterility. The testes of three of the male steriles that were not demonstrated to be T(X;Y;A)'s were similarly examined. Two of them exhibited the failure of sperm head elongation characteristic of T(X;A)'s, and the third seemed to be normal. Further examination of males of this stock revealed that, despite their sterility, they do produce motile sperm but the viability of the males is extremely low, and general debility may account for their failure to reproduce.

Seven of the 17 cytologically detectable T(X;Y;A)'s contained two independent translocations with one chromosomal element in common. In five, the X was the shared element [i.e., they were T(X;A) + T(X;Y)], and all five were male sterile. In another male sterile case, chromosome 2 was the shared element [i.e., T(X;2) + T(Y;2)]; since this is the only translocation recovered that could be demonstrated not to involve a T(X;Y), it has been eliminated from Table 3. The last case was male lethal, and the Y was the shared element; i.e., T(X;Y) + T(Y;A). The remaining 10 T(X;Y;A)'s were cyclic translocations, i.e., Y^DX^P; A^DY^P; X^DA^P or A^DX^P; X^DY^P; Y^DA^P. The sample of 10 cyclic translocations contained nine (2 lethal + 7 sterile) of the first type and one (lethal) of the second type. We have no explanation of this astonishing inequality. It is possible that the two types of cyclic translocations are not produced with equal frequency, or, as seems more likely at present, that they are produced but not recovered with equal frequency.

Of the 43 T(X;Y)/Y male fertile translocations, only 14 are fertile as T(X;Y)/0 males, and the remaining 29 are sterile. Fifteen of the 29 sterile cases are broken in the proximal X heterochromatin and are presumably sterile because they have lost half of the translocation; i.e., Y^DX^P with its associated Y fertility factors. Eliminating these from the T(X;Y)/Y fertile sample we are left with 14 fertile and 14 sterile as T(X;Y)/0. This value agrees well with the 50 percent frequency of inactivation of Y fertility genes found among T(Y;A)'s (NEUHAUS 1939; BROSSEAU, unpublished). All 14 of the inactivations of Y

chromosome fertility factors are associated with breaks in Y^L and presumably involve *KL*. BROSSEAU (1960), on the other hand, found that, among 50 losses of fertility factors that were not demonstrably associated with translocations, 37 involved the *KL* complex and 13 involved *KS*. We can offer no explanation for this discrepancy.

Whereas the sample of translocations investigated has numerous cases with breaks in the chromocentral region of the X (19/86), the subsample that is male lethal is characterized by euchromatic break points (20/22). One of the two lethal T(X;Y)'s with a heterochromatic X chromosome break point has an inversion of the X with a euchromatic break point associated with the T(X;Y). Neither of these translocations survives in combination with *Yma-l*$^+$, a Y chromosome that carries the proximal heterochromatic-euchromatic region of the X (BROSSEAU *et al.*, 1961); we have therefore concluded tentatively that the lethality of these two translocations is not associated with their heterochromatic X chromosome break points. The scarcity of lethals associated with proximal heterochromatic break points is understandable in view of the homology shared by Xh and the Y; lethals in Xh would be covered by the homologous region of the translocated Y, as well as by the extra normal Y with which they are recovered.

The two translocations that were T(X;Y)/Y but not T(X;Y)/0 viable were from the T(X;Y)/Y male sterile group and were actually T(X;Y;A)'s. One was a cyclic translocation with the X broken at 15E and 3L broken in 74; the other was a T(X;3) with the X chromosome break point at 3F and 3L broken in 69 plus a T(X;Y) with the X break point at 12A. Since these translocations are lethals whose expression is suppressed by an extra Y chromosome, it is likely that they are variegated position effect lethals (LINDSLEY *et al.* 1960). It is interesting to note that the T(Xh;Y^S)'s that are male sterile in the absence of a normal Y and are assumed to be sterile because of the loss of Y^DX^P (i.e., to be X^DY^P/0) are able to survive without a normal Y. This indicates that the X^DY^P element is not deficient for the Xh genes required for survival, e.g., *bb* and the nucleolus organizer. That is to say the break in X is proximal to *bb* and *NO* or the break in Y^S is distal to *bb* and *NO*. In view of BAKER's finding (1955, 1957) that the breakage distance between *bb*$^+$ and the Y centromere is very small, it seems likely that the second alternative is more often the correct one.

DISCUSSION

Of the 87 translocations originally selected, 86 involved an interchange between the X and the Y chromosomes. Since the translocations were obtained from the daughters of irradiated males, it was possible to recover male lethal and male sterile translocations. Of the 86 T(X;Y)'s recovered, 22 were lethal and 21 were sterile as T(X;Y)/Y males. Had we chosen to recover our translocations from T(X;Y)/Y sons rather than T(X;Y)/X daughters of irradiated males, our sample would have been halved. Further investigation of the T(X;Y)/Y viable and fertile cases revealed that 14 were fertile and 14 were sterile as T(X;Y)/0; 15

others that had the X chromosome break point in the proximal heterochromatin were sterile, presumably because they had lost the $Y^{p}X^{p}$ half of the translocation. It therefore seems likely that, if we had chosen to recover translocations from T(X;Y)/0 sons of the irradiated males, we would have obtained a sample only about one fourth as large as that obtained from T(X;Y)/X females. It is also true that, had a Y chromosome marked on both ends been used in the present experiments in place of the singly marked Y's, we would probably have recovered an additional 45 translocations that were missed in this experiment [i.e., $T(Xh;Y^{L})$].

From the preceding paragraph it is clear that a valid sample of rearrangements involving either of the sex chromosomes cannot be recovered from sons of irradiated parents. Yet there are several published instances in which such a sample of rearrangements was assumed to be a representative one, and erroneous conclusions were drawn concerning the frequency of sex chromosome involvement in rearrangements, even though the data on which the conclusions were based were often in disagreement with those of BAUER *et al.* (1938) and BAUER (1939) who had, except for wholly heterochromatic rearrangements, as nearly an unbiased sample of aberration associated breaks as can be achieved. GLASS (1955) concluded that the Y chromosome is involved in translocation less than one third as frequently as either major autosome. Similarly, BAKER (1949) concluded for *Drosophila virilis* that the frequency with which the Y chromosome is involved in translocations is less than half as great as any of the autosomes. HAAS, DUDGEON, CLAYTON, and STONE (1954), also working with *D. virilis*, go even further and conclude that recovery of fewer translocations involving the Y than expected on the basis of metaphase length shows greater rearrangement frequency for euchromatin than heterochromatin. KIRSSANOV (1946), on the other hand, realized that the frequent male sterility of T(Y;A)'s observed by NEUHAUS (1939) would bias a sample of such translocations recovered in males. Correcting for the sterility, he concluded that the frequency with which the Y chromosome is involved in translocations is proportional to its metaphase length, as had previously been postulated by BAUER *et al.* (1938).

Recovery of X chromosome rearrangements may be similarly biased by sex-linked lethality as well as sterility. This effect of male inviability was recognized by PATTERSON *et al.* (1934) as the reason that they were not able to recover as many T(X;2) + T(X;3)'s as T(2;3)'s, but the contribution of male sterility to this inequality seems not to have been recognized. GLASS (1955) recovered X-autosome translocations from daughters of irradiated males. The two X chromosomes of these females, however, were of the same sequence and not well marked. His reduced recovery of T(X;A)'s was therefore caused by his inability to recognize many of them rather than by less frequent involvement of the X in translocations, as he erroneously concluded.

The correlation between chromosome length, as measured by breakage and as measured by other criteria, has been the subject of a good deal of experimental work (BAUER *et al.* 1938; BAUER 1939; KAUFMANN 1946, e.g.). As inferred here, the conclusion has been that breakage length roughly parallels metaphase length, at least to the extent that the apportionment of breaks among euchromatic and

proximal heterochromatic regions of the genome approximates the ratio of their lengths. Our findings are roughly in agreement with this relationship since the ratio of Xe to Xh breaks in the T(X;Y^s)'s is 20 to 19, and, according to COOPER (1959), Xh comprises about half of the metaphase X. Furthermore, among the T(Xe;Y)'s, the ratio of Y-arm involvement is T(Xe;Y^L):T(Xe;Y^s) = 47:20 and estimates of the ratios according to metaphase length run from 1.5 to 2.

The sample of translocations investigated contained 64 male viable cases of which 47 had euchromatic X chromosome break points. We had anticipated that this type of rearrangement would frequently be associated with variegated position effect. In no case, however, was it possible to observe any phenotype, variegated or otherwise, in either T(X;Y)/Y or T(X;Y)/0 males. One male lethal case, in which the X was broken in region 3C, exhibited a variable Notch phenotype in T(X;Y)/FM4 females, whereas the same phenotype was not observed in T(X;Y)/Y^{s}X·Y^L females. This is the only possible instance of a V-type position effect with a phenotype other than inviability that we observed, although there were two cases that were T(X;Y)/0 but not T(X;Y)/Y lethal. In both these cases an autosome was also involved in the translocation, but the only heterochromatic break point was in the Y chromosome, and it seems reasonable to conclude that it is the transfer of X euchromatic loci into the Y heterochromatin that is responsible for the lethality. Some of the translocations that were T(X;Y)/Y inviable may also be variegated position effects whose expression is so extreme that the suppressing effect of an extra Y chromosome is not sufficient to allow them to survive. This type of variegated lethality has not been demonstrated, but LINDSLEY *et al.* (1960) have shown that, of the sex-linked lethals induced by 3 and 4 kr that die as X/0 males, about 19 percent survive as X/Y males, but only an additional three percent survive as X/Y/Y males.

It is known that Y;A rearrangements can be associated with variegated position effects, because such rearrangements have been recovered in experiments in which variegated phenotype was the selective criterion. KHVOSTOVA (1939) found 19/192 *R(ci$^+$)*'s were associated with T(Y;4)'s; also SLATIS (1955) and MICKEY (1959) report position effects at the *bw* locus associated with T(Y;2)'s. It is worthy of note, however, that PANSHIN (1938; cited by LEWIS 1950) found one of eight induced reversals of the T(1;4)*w^{m11}* phenotype to be associated with the transfer of the tip of the X from the short arm of chromosome 4 to the Y chromosome.

It is difficult to decide whether T(Xe;Y)'s represent an abnormal sample of euchromatin-heterochromatin realignments with respect to variegation frequency or the present sample provides a reasonable measure of the frequency with which euchromatic genes produce a variegated phenotype when they are brought into juxtapostion with heterochromatin.

SUMMARY

A sample of 86 T(X;Y)'s was recovered from irradiated X/Y sperm and a cytological and genetic analysis performed. The results of this analysis are pre-

sented in Table 3. Several points of interest have emerged: (1) The distribution of break points between heterochromatin and euchromatic regions as well as between Y arms is roughly proportional to metaphase length. (2) The distribution of break points in the X euchromatin indicates preferential breakage in some regions (3, 7, 11), at least in translocations involving Y^L. (3) Translocations that are T(X;Y)/Y lethal preponderantly involve euchromatic breaks in the X, presumably because heterochromatic lethals are covered by the Y chromosome. (4) Translocations that are T(X;Y)/Y sterile, i.e., not made fertile by the presence of a normal Y, are nearly all T(X;Y;A)'s. (5) No translocation that is T(X;Y)/Y viable and fertile is associated with a T(X;Y;A). (6) Among cyclic T(X;Y;A)'s, interchanges that are Y^DX^P; A^DY^P; X^DA^P are found much more frequently than those that are A^DX^P; X^DY^P; Y^DA^P. (7) Translocations that are T(X;Y)/Y fertile but T(X;Y)/0 sterile (i.e., made fertile by the presence of a normal Y) seem to involve inactivation of fertility factors in Y^L to the exclusion of Y^S. (8) Only three possible cases of variegated type position effect were encountered among the 86 translocations.

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LITERATURE CITED

Abrahamson, S., I. H. Herskowitz, and H. J. Muller, 1956 Identification of half translocations produced by X-rays in detaching attached-X chromosomes of *Drosophila melanogaster*. Genetics **41**: 410–419.

Baker, W. K., 1949 The production of chromosomal interchanges in *Drosophila virilis*. Genetics **34**: 167–193.

1955 On the structure of the *sc*^{s}Y:*bw*+ chromosome of *Drosophila melanogaster*. Drosophila Inform. Serv. **29**: 101–102.

1957 Induced loss of a ring and a telomeric chromosome in *Drosophila melanogaster*. Genetics **42**: 735–748.

Bauer, H., 1939 Röntgenauslösung von Chromosomenmutationen bei *Drosophila melanogaster*. I. Bruchhäufigkeit, -Verteilung und -Rekombination nach Speicheldrüsenuntersuchung. Chromosoma **1**: 343–390.

Bauer, H., M. Demerec, and B. P. Kaufmann, 1938 X-ray induced chromosomal alterations in *Drosophila melanogaster*. Genetics **23**: 610–630.

Bridges, C. B., 1938 Revised salivary map of X chromosome of *Drosophila melanogaster*. J. Heredity **29**: 11–13.

Brosseau, G. E., Jr., 1958 Crossing over between Y chromosomes in male Drosophila. Drosophila Inform. Serv. **32**: 115–116.

1960 Genetic analysis of the male fertility factors on the Y chromosome of *Drosophila melanogaster*. Genetics **45**: 257–274.

Brosseau, G. E., Jr., B. Nicoletti, E. H. Grell, and D. L. Lindsley, 1961 Production of altered Y chromosomes bearing specific sections of the X chromosome in Drosophila. (In press)

CATSCH, A., 1948 Versuche an Drosophila über die Dosisabhängigkeit strahlenduzierter Chromosomenmutationen. Z. Ind. Abst. Vererb. **82**: 155–163.

COOPER, K. W., 1959 Cytogenetic analysis of major heterochromatic elements (especially Xh and Y) in *Drosophila melanogaster*, and the theory of "heterochromatin." Chromosoma **10**: 535–588.

GLASS, B., 1955 A comparative study of induced mutation in the oocytes and spermatozoa of *Drosophila melanogaster*. I. Translocations and inversion. Genetics **40**: 252–267.

HAAS, F. L., E. DUDGEON, F. E. CLAYTON, and W. S. STONE, 1954 Measurement and control of some direct and indirect effects of X-radiation. Genetics **39**: 453–471.

KAUFMANN, B. P., 1946 Organization of the chromosome. I. Break distribution and chromosome recombination in *Drosophila melanogaster*. J. Exptl. Zool. **102**: 293–320.

KHVOSTOVA, V. V., 1939 The role of the inert regions of chromosomes in the position effect of the cubitus interruptus gene in *Drosophila melanogaster*. Bull. Acad. Sci. URSS, Sér. biol. 541–574.

KIRSSANOV, B. A., 1946 Frequency of breaks of heterochromatin during the translocation process in *Drosophila melanogaster*. I. Frequency of breaks in the Y chromosome. Izvest. Akad. Nauk SSSR Ser. Biol. **5**: 501–518.

LEWIS, E. B., 1950 The phenomenon of position effect. Advances in Genet. **3**: 73–115.

LINDSLEY, D. L., C. W. EDINGTON, and E. S. VON HALLE, 1960 Sex-linked recessive lethals in Drosophila whose expression is suppressed by the Y chromosome. Genetics **45**: 1649–1670.

MICKEY, G. H., 1959 Observation on variegated position effects in *Drosophila melanogaster*. Univ. Texas Publ. **5914**: 99–105.

MISLOVE, R. F., and E. B. LEWIS, 1954 Report of Rhoda F. Mislove and E. B. Lewis. Drosophila Inform. Serv. **28**: 77.

MULLER, H. J., 1940 An analysis of the process of structural change in chromosomes of Drosophila. J. Genet. **40**: 1–66.

1948 The construction of several new types of Y chromosomes. Drosophila Inform. Serv. **22**: 73–74.

MULLER, H. J., and I. H. HERSKOWITZ, 1954 Concerning the healing of chromosome ends produced by breakage in *Drosophila melanogaster*. Am. Naturalist **88**: 177–208.

NEUHAUS, M. E., 1939 A cytogenetic study of the Y chromosome of *Drosophila melanogaster*. J. Genet. **37**: 229–254.

NICOLETTI, B., and D. L. LINDSLEY, 1960 Cytogenetic analysis of T(X;Y)'s. Drosophila Inform. Serv. **34**: (In press).

PANSHIN, I. B., 1938 The cytogenetic nature of the position effect of the genes white (mottled) and cubitus interruptus. Biol. Zhur. **7**: 837–865.

1941a Experimental proof of non-union of chromosome fragments at the location of reciprocal translocations. Compt. Rend. Acad. Sci. URSS **30**: 438–443.

1941b Relative frequency of union of chromosome fragments. Compt. Rend. Acad. Sci. URSS **33**: 320–322.

PARKER, D. R., 1953 Observation on X-ray induced detachments of attached-X chromosomes in Drosophila. J. Tenn. Acad. Sci. **28**: 185.

1954 Radiation induced exchanges in Drosophila females. Proc. Natl. Acad. Sci. U.S. **40**: 795–800.

PARKER, D. R., and A. E. HAMMOND, 1958 The production of translocations in Drosophila oocytes. Genetics **43**: 92–100.

PARKER, D. R., and J. MCCRONE, 1958 A genetic analysis of some rearrangements induced in oocytes of Drosophila. Genetics **43**: 172–186.

PATTERSON, J. T., W. STONE, S. BEDICHEK, and M. SUCHE, 1934 The production of translocations in Drosophila. Am. Naturalist **68**: 359–369.

PROKOFYEVA-BELGOVSKAYA, A. A., 1937 The structure of the Y chromosome in the salivary glands of *Drosophila melanogaster*. Genetics **22**: 96–103.

SCHULTZ, J., 1947 The nature of heterochromatin. Cold Spring Harbor Symposia Quant. Biol. **12**: 179–191.

SLATIS, H. M., 1955 Position effect at the brown locus in *Drosophila melanogaster*. Genetics **40**: 5–23.

STERN, C., 1927 Ein genetischer und zytologischer Beweis für Vererbung im Y Chromosom von *Drosophila melanogaster*. Z. Ind. Abst. Vererb. **44**: 187–231.

SEGREGATION IN NEW ALLOPOLYPLOIDS OF NICOTIANA. I. COMPARISON OF 6x (N. TABACUM × TOMENTOSIFORMIS) AND 6x (N. TABACUM × OTOPHORA)

D. U. GERSTEL[1]

North Carolina State College, Raleigh, North Carolina

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GENETIC segregation in synthetic allopolyploids of the genus Gossypium has been described in several earlier papers by the author and his colleagues (see GERSTEL and PHILLIPS 1958, for a summary). We had found in that genus that allopolyploids produced from two very closely related species segregated like autopolyploids, i.e. they gave approximately 5:1 ratios for crosses of the duplex type *ZZzz* to recessive testers. In contrast, very little segregation occurred where the two parents of an amphiploid belonged to different sections of the genus; such amphiploids behaved like classical allopolyploids. From still other amphiploids intermediate ratios had been obtained. In some amphiploids the ratios differed somewhat from locus to locus, but their averages reflected in a quantitative fashion the taxonomic affinity of the two parent species which were combined in the particular amphiploid. Herein lay the main interest of the method, specially when the argument was inverted and the relationship between two species assessed quantitatively from the segregation frequencies of their amphiploid.

This paper commences a series of similar studies on segregation in synthetic allopolyploids of the genus Nicotiana.

MATERIALS AND METHODS

The three species of Nicotiana used were the diploid ($n = 12$) species *N. tomentosiformis* Goodsp., *N. otophora* Griseb. and the natural tetraploid *N. tabacum* L. Two accessions were employed of each of the diploids. The *N. tomentosiformis* accessions had been given to us by the late PROFESSOR R. E. CLAUSEN of the University of California and came originally from the locations Cañamina and Tajma in Bolivia; we also owe the Jujuy (Argentina) race of *N. otophora* to the courtesy of DR. CLAUSEN. A second accession of *N. otophora* had been obtained from U. S. Department of Agriculture sources. Though these accessions had been maintained for many years in the U. S. by close inbreeding, the Tajma race still segregated for the factor *Pb*, or purple bud, of which use was made as a marker; both *N. otophora* accessions were homozygous for purple bud. The symbol *Pb* will also be used for the *N. otophora* factor for convenience though homology of the purple bud factors from the two diploid species was not proven.

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Purple bud is unknown in *N. tabacum*; the character behaved as a dominant in interspecific hybrids.

The remaining markers employed were recessive mutants of *N. tabacum*; here also the use of identical symbols for the dominant genes derived from the wild species does not necessarily imply homology. The mutants coral *(co)*, fasciated *(fs)*, and white seedling *(ws)* were available in the otherwise, more or less, isogenic background of the race Red Russian and were obtained from DR. CLAUSEN's collection. Fasciated plants and white seedlings differ from some normal varieties by two recessive factors (CLAUSEN and CAMERON 1950 and unpublished), but single dominant genes from the diploid species cover the recessives completely, and the segregation of these single dominant alleles was studied. The marker white corolla *(wh)* came from the tobacco race known as Cuba White. Burley tobacco (varieties Ky 16 and By 21) is characterized by white stems and early yellowing leaves; it differs by two recessives *(yb_1 yb_2)* from most normal tobacco varieties (STINES 1959), but a single dominant gene from the diploids produces fully normal plants. Yellow green *(yg)* had originally been found by NOLLA (1934), but was employed here in the local variety "402" into which DR. T. J. MANN had transferred the *yg* allele.

Amphiploids were produced from F_1 seed of interspecific hybrids by treating emerging seedlings at room temperature with 0.1 percent aqueous colchicine solution for three hours. Positive results were achieved almost without fail; but in addition to the desired amphiploids the cultures usually contained unaffected plants, periclinal chimaeras with polyploid morphology but low fertility, offtypes (probably aneuploids) and monstrosities of various degrees (probably at least in part higher polyploids). All these were discarded and only plants of typical morphology, high percentage of filled pollen and good spontaneous seed set were used. These plants were presumably of the duplex genotype *ZZzz*, where the dominant alleles had been derived from the wild parent and the recessives from *N. tabacum*. Since the chromosome number was checked only rarely, it is quite possible that some of the amphiploids were numerically not fully balanced. Influence upon segregation from that source was minimized by testing several amphiploids of the same origin wherever possible. The segregation ratios were obtained by crossing the amphiploids with *N. tabacum* lines homozygous for the appropriate recessive marker. The observed segregation frequencies, therefore, reflect directly the gametic output of the amphiploid parents. An exception were the tests involving *Ws*. Since white seedling plants were inviable, amphiploids had to be synthesized from green heterozygotes (actually *ws_1 ws_1 Ws_2 ws_2*) and wild species. About one half of these amphiploids segregated and the other half did not; the latter were discarded. The crosses designed to test segregation were made between the segregating amphiploids (for short *Ws Ws ws ws*) and *Ws ws* heterozygotes of *N. tabacum*. The observed segregation ratios were then corrected by dividing the total offspring minus twice the number of white seedlings by twice the number of white seedlings.

Segregation for the white seedling character was scored from seedlings germinating in Petri dishes. The other characters were scored from older plants.

RESULTS

6x *(N. tabacum* × *tomentosiformis):* In the first column of Table 1 the marker genes and in the second column the assigned plant numbers of the individuals employed are listed. In most cases some replication was available, either from two or more individual amphiploids, or from different accessions of *N. tomentosiformis* (third column) or from reciprocal crosses (fourth column). In the case of *Fs* no replication was obtained, and for *Wh* the data from three parents were not homogeneous ($P < .01$), and their summation is not justified. One would suspect that plant B 53-2 which gave an approximate 1:1 ratio was an unbalanced type, perhaps *Wh wh wh* instead of *Wh Wh wh wh.*

The over-all picture of the ratios (last column) is this: within each genotype the results are quite uniform since reciprocal testcrosses and the data from the two accessions of *N. tomentosiformis*, as available, gave similar results. Generally, the ratios are smaller than the tetrasomic 5:1 expected with random chromosome segregation; in most cases the ratios are even smaller than 3.7:1 which could result from random chromatid segregation, though only in one case(*) significantly so. Only the ratios for *Yg* were larger than 3.7:1.

In all of the progenies only two phenotypes could be separated with certainty. With incomplete dominance or pronounced dosage effects it might have been possible to distinguish between three classes from the crosses which were *ZZzz* × *zz* as had been possible in the Gossypium work quoted above. In the material segregating for *Fs* the 29 plants scored as having the recessive *fs* phenotype showed more or less extreme fasciation of the stem and had at least four carpels to each pistil; the remaining 87 plants had normal vegetative growth and usually the normal number of two carpels per pistil; a number of plants in this class had an occasional flower with three carpels. Three plants were intermediate; they could have been chromosomally unbalanced.

Presence or absence of anthocyanin coloration of the calyces and pedicels *(Pb* vs. *pb)*, best studied in flower buds, was easily verified though the intensity of coloration was variable. Two of the families segregating for *Pb* segregated simultaneously for *Wh*. These are listed separately in Table 2. Instead of the expected two classes with white and pink corollas, three classes were found; among the *Pb* plants 12 had very pale pink flowers, but purely white ones were missing. Apparently the *Pb* factor produces a flush of color in the *wh* flowers. Listed in Table 2 is also the progeny from amphiploid B 53-2, which did not carry *Pb* and in the offspring of which no very pale flowered plants appeared.

Plants B 51-17 and B 55-15 were amphiploids from burley tobacco and *N. tomentosiformis* carrying *Pb*; the backcross populations to burley segregated simultaneously for *Pb* and *Yb*. Forty-eight of the total offspring were phenotypically *Yb Pb*, 20 were *yb pb*, one was *Yb pb* and two *Yb* plants did not reach the flowering stage. Thus the nonburley and purple bud factors are linked in *N. tomentosiformis*. CLAUSEN and CAMERON (1944) had previously shown by means of monosomic analysis that yb_1 and a purple plant factor transferred from

TABLE 1

Segregation of 6x (N. tabacum × tomentosiformis)

Character	Plant no. of hexaploid	Tomentosiformis strain in hexaploid	Hexaploid as	Phenotype of progeny		Ratio
				Dominant	Recessive	
Fs	C 114–8	Cañamina	♂	87	29	3.0:1
Pb	B 48–11	Tajma	♀	66	18	
	B 53–1	Tajma	♀	30	10	
	B 53–4	Tajma	♀	51	16	
				147	44	3.3:1
	B 48–11	Tajma	♂	39	17	
	B 51–17	Tajma	♂	34	17	
	B 53–4	Tajma	♂	284	67	
	B 54–5	Tajma	♂	4	0	
	B 54–12	Tajma	♂	6	9	
	B 55–15	Tajma	♂	14	4	
				381	114	3.3:1
Wh	B 53–1	Tajma	♀	32	5	
	B 53–2	Tajma	♀	26	30	
	B 53–4	Tajma	♀	56	11	
				(114	46	2.5:1)
	C 117–2	Cañamina	♂	226	97	2.3:1*
Ws	C 121–3	Cañamina	♀	762	95	3.5:1†
	C 121–3	Cañamina	♂	2481	309	3.5:1‡
Yb	A 25–A	Cañamina	♀	143	49	
	A 25–D	Cañamina	♀	171	51	
				314	100	3.1:1
	A 25–A	Cañamina	♂	70	19	
	A 25–D	Cañamina	♂	86	34	
				156	53	2.9:1
	B 51–17	Tajma	♂	36	16	
	B 55–15	Tajma	♂	15	4	
				51	20	2.6:1
Yg	C 120–4	Cañamina	♀	107	23	4.7:1
	B 54–5	Tajma	♀	198	45	4.4:1

* Highly significant minus deviation from 3.7:1.
† Ratios corrected since a *Ws ws* tester had to be used.
‡ One subfamily gave a heterogeneous 186:8 (or 11.1:1 corrected) ratio and was omitted from the total.

TABLE 2

Simultaneous segregation for Pb *and* Wh *in 6x* (N. tabacum × tomentosiformis)

Hexaploid parent (♀)	Corolla color	Bud color: Purple: Pink	Purple: Very pale	Purple: White	Green: Pink	Green: Very pale	Green: White
B 53–1*		26	2	0	6	0	3
B 53–2†		Not segregating for *Pb*			26	0	30
B 53–4		41	10	0	15	0	1

* Two additional plants were scored *Pb* and one *pb*, but came into bloom too late in the season for the corolla color to be recognized.
† This family is listed here because it shows that in the absence of *Pb* no very pale flowers are obtained.

the related species *N. tomentosa* were both located in the same tobacco chromosome.

6x *(N. tabacum* × *otophora):* Segregation ratios for crosses from this combination were in each case larger than in the progenies from 6x *(N. tabacum* × *tomentosiformis)* scored for similar loci (Table 3). This is the only generalization which can be drawn from the data presented in the last column. The ratios for different loci cover a wide range from 3.5:1 to 14.8:1, and the results from replications, where available, are not in as good an agreement as for 6x *(N. tabacum* × *tomentosiformis).* Further work with the 6x *N. tabacum* × *otophora* combinations is needed in order to clear up the reasons for the lack of uniformity of the results.

Several points are of interest. As in the preceding chapter, *Yg* gave the widest ratio of all the loci which were studied. With respect to the *Co* and *Ws* genes somatic instability was observed. Among the offspring of plant B 22-3 which segregated for *Co* 12 segregants had self-coral flowers. One hundred and nineteen of the 128 plants scored as carmine had variegated carmine-coral flowers while in eight no variegation could be detected. The extent of variegation differed from occasional small coral spots on carmine background (never *vice versa*) to heavy sectoring. It is not impossible that the eight self-carmine plants also fell into the variegated category and that the spots were too infrequent and too small to be observed with the naked eye, though numerous flowers were studied from each of these plants. One additional plant was not scored for variegation since it bloomed late in the season, but it could be classified as carmine from its flower buds.

Variegation was also observed among the *Ws* material from plants D 134-4 and D 134-6. Ninety-eight seedlings were albinos; among the 1887 seedlings scored as green 12 were found with larger or smaller sectors on their cotyledons lacking chlorophyll (Figure 1). Five of these plants were grown to a later stage; they continued to produce variegated leaves. The seedlings with self-green cotyledons and the albinos were discarded after scoring at the cotyledonary stage. Among the green progeny of plant C 107-13 no variegation was observed on the cotyledons. The green seedlings of one subfamily were transferred to the field;

TABLE 3

Segregation of 6x (N. tabacum × otophora)

Character	Plant no. of hexaploid	Otophora strain in hexaploid	Hexaploid as	Phenotype of progeny		Ratio
				Dominant	Recessive	
Co	B 22–3	Jujuy	♂	128	12	10.7:1
Fs	B 32–2	USDA	♀	308	60	5.1:1
	B 32–2	USDA	♂	195	56	3.5:1
Pb	B 32–2	USDA	♀	329	39	8.4:1
	A 5–D	USDA	♂	68	14	
	A 9–E	USDA	♂	72	9	
	B 32–2	USDA	♂	202	54	
				342	77	4.4:1
	B 22–3	Jujuy	♂	108	32	
	B 23–3	Jujuy	♂	17	4	
	C 107–13	Jujuy	♂	165	30	
				290	66	4.4:1
Ws	D 134–4	USDA	♀	126	3	
	D 134–6	USDA	♀	1761	95	
				1887	98	9.1:1†
	C 107–13	Jujuy	♂	644	44	6.8:1†
Yb	A 5–D	USDA	♀	159	19	8.4:1
Yg	A 9–C	USDA	♀	260	24	
	A 9–D	USDA	♀	239	12	
	A 9–E	USDA	♀	286	17	
				785	53	14.8:1

† Ratios corrected since a *Ws ws* tester had to be used.

of 200 survivors two were found to be chlorophyll variegants. Thus somatic instability with respect to chlorophyll occurred in derivatives of amphiploids with *N. otophora* from both Jujuy and U.S.D.A. accessions.

DISCUSSION

N. tabacum is the classic example of a natural amphiploid. The mode of its origin was demonstrated in 1928 by GOODSPEED and CLAUSEN, who showed that one of the diploid ancestors came from the section Tomentosae to which both *N. tomentosiformis* and *N. otophora* belong. For short. we may designate these as the T species and the corresponding subgenome of tobacco as the T genome. It is quite likely that none of the now living species of that taxonomic group is identical with the parent that once went into ancestral *N. tabacum*, but perhaps it is not entirely futile to attempt to designate the modern species closest to that

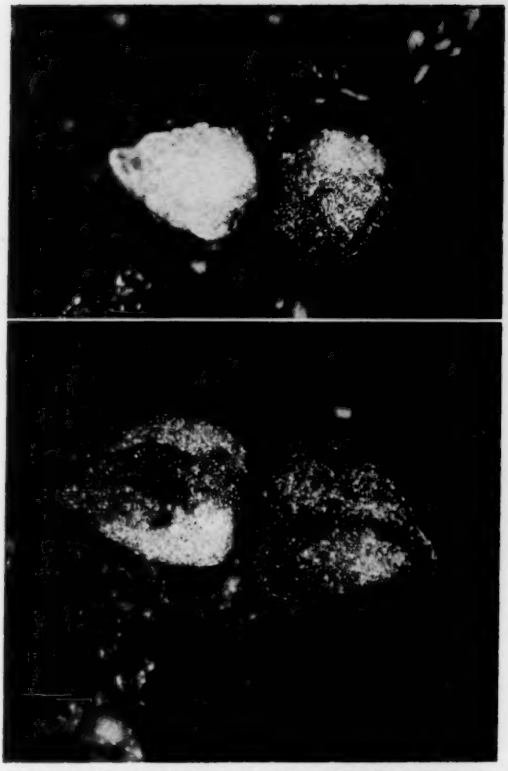

FIGURE 1.—Seedlings showing instability of the chlorophyll factor from *N. otophora* (Approx. 24×).

ancestor. In the literature various arguments can be found favoring *N. tomentosiformis* over *N. otophora* or *vice versa*. Support for *N. otophora* comes from two observations. This species forms with *N. sylvestris* an amphiploid which is both male and female fertile, at least to some degree. *N. sylvestris* is believed by CLAUSEN and GOODSPEED to represent the other ancestral genome ("S") of tobacco. Amphiploids between other species of the Tomentosae and *N. sylvestris* are completely female sterile; the nature of these sterilities has been analyzed, and it has been found that relatively simple complementary gene systems are involved in each case (GREENLEAF 1942; AR-RUSHDI 1956). Secondly, only *N. otophora* and *N. sylvestris* share at present an extended geographic range between Salta and Sucre on the eastern slopes of the Andes; the other Tomentosae occur further north where *N. sylvestris* has not been found (see maps in GOODSPEED 1954). On the debit side *N. otophora* possesses, besides other morphological peculiarities, strongly reflexed corolla lobes (Figure 3a) which are retained in the

FIGURE 2.—Plant raised from a seedling of the type shown in Figure 1 with continuing instability.

amphiploid *N. sylvestris* × *otophora* (Figure 3b) but cannot be found in tobacco (Figure 3c). In contrast the floral morphology of *N. tomentosiformis* (Figure 3d) is much more like that of tobacco, and the *N. tomentosiformis* × *sylvestris* amphiploid resembles *N. tabacum* closely.

Thus, distribution and fertility point towards *N. otophora* as the closer relation of tobacco while morphology decidedly favors *N. tomentosiformis*. It is of course possible, as AR-RUSHDI (1956) suggested, that early hybridization between the various T species combined in a single segregant form the various features which could have made it a successful ancestor of tobacco. Since, thus far, hybridization and introgression between T species has not been demonstrated, AR-RUSHDI's viewpoint must be considered speculative for the time being. It may be noted, however, that GOODSPEED (1954, p. 365) mentions the possibility that in the origin of *N. tomentosiformis* hybridization of *N. tomentosa* and *N. otophora* may have been involved.

Does the segregation of artificial polyploids which in Gossypium fairly faithfully reflected the relation between the combined species provide any clue to discriminate between *N. tomentosiformis* and *N. otophora* as the closer modern relative of tobacco? Quite consistently *N. tabacum* × *tomentosiformis* amphiploids gave smaller segregation ratios than the *N. otophora* combinations. This consistency points towards a greater homology of *N. tomentosiformis* chromosomes with those of the T subgenome of tobacco. But certain difficulties were

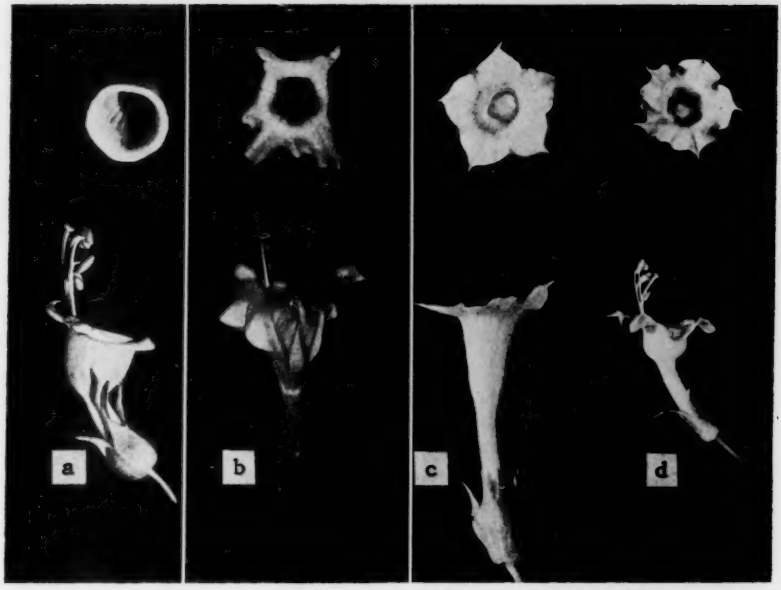

FIGURE 3.—Flowers of (a) *N. otophora,* (b) amphiploid *N. sylvestris* × *otophora* (first made by R. E. CLAUSEN), (c) *N. tabacum,* (d) *N. tomentosiformis* (Approx. natural size).

encountered in the Nicotiana work which were absent from the previous studies with Gossypium, and until these are fully understood judgment must be withheld.

In 6x *(N. tabacum* × *tomentosiformis)* all observed ratios were smaller than the 5:1 gametic output characteristic of autopolyploids. This could perhaps be ascribed to chromatid, as opposed to chromosome segregation. However, the chromatid segregation ratio of 3.7:1 is a limiting value obtained only under special conditions, one of which is great distance between centromere and locus on the chromosome (see review by LITTLE 1945). It is, therefore, surprising to find such low segregation ratios for almost all the loci which were studied. For most segregations in Table 1 the observed ratios were below those obtainable from chromatid segregation and in the starred case even significantly so. These results bring to mind the example of SKALINSKA (1935) who obtained a large excess of recessives from a selfed amphiploid *Aquilegia chrysantha* × *flabellata.* Instead of the 35:1 ratio expected from selfing of an autotetraploid, SKALINSKA observed an almost exact 3:1 ratio of 125 blue to 43 white flowering plants. That author reached the conclusion that at the first meiotic anaphase the two chromosomes bearing *C* (colored) always disjoined from those with *c* (white) resulting in only two kinds of gametes *(CC* and *cc)* giving in turn a 3:1 F_2 ratio. This phenomenon seems to resemble the "affinity" recently postulated by MICHIE (1953) and WALLACE (1958) for chromosomes of interspecific and interracial

hybrids of mice. WALLACE proposed that at first meiotic division centromeres of the same ancestral origin in such hybrids tend to travel to the same pole. The difference consists in that in the mouse material non-homologous chromosomes showed affinity, whereas in the Aquilegia amphiploid homologues of common origin were postulated to go to the same meiotic pole.

SKALINSKA's main argument was a negative one, namely that numerical irregularities in chromosome distribution were excluded as a cause of her results because all the progeny plants which she had studied possessed the full tetraploid chromosome number of 28. In the Nicotiana amphiploids described in this paper affinity may play a role, but it is not unlikely that genetic instability, either chromosomal or mutational, is a factor responsible for an increase of the frequency of recessive offspring by transforming potential *Aa* gametes into *aa* or *a*.

Observations to support this viewpoint have not yet been made with the critical *N. tabacum* × *tomentosiformis* amphiploids which gave the unusual ratios, but the literature on genetic instability in Nicotiana is fairly extensive. This was recently reviewed by MOAV and CAMERON (1960) who demonstrated such instability in combinations of *N. tabacum* and *N. plumbaginifolia*. Despite the distant relationship of these two species their amphiploid segregated extensively for a factor for which it was of duplex constitution. Since instability for this factor had been amply demonstrated by the authors, they attributed the appearance of recessives in the progeny to the same cause.

In addition to the unstable hybrids between distantly related species listed by MOAV and CAMERON (1960), SMITH and SAND (1957) described cases of flower color variegation in hybrid derivatives from closely related *N. langsdorffii* and *N. sanderae*. The unstable pentaploids derived from 6x *(N. tabacum* × *otophora)* mentioned in the present paper are additional examples of instability in hybrids from closely related parents. Studies in progress are expected to reveal whether there is a relation between the somatic instabilities found and the progeny ratios which were obtained.

The unusual but fairly consistent ratios from 6x *(N. tabacum* × *tomentosiformis)* do not represent the only problem calling for a solution. The ratios from the *N. tabacum* × *otophora* amphiploids were inconsistent and varied over a wide range from 3.5:1 for *Fs* to 10.7:1 for *Co* (P for homogeneity $< .001$) omitting from consideration the even larger ratio for *Yb* for reasons given below. This spread may reflect variable amounts of preferential pairing of different chromosomes but could also have other causes, as, e.g., differences in the degree of instability of the various markers. In this context it may be noted that the *Co* factor from *N. otophora* was somatically unstable in almost every plant of the pentaploid progeny whereas *Ws* from the same source was unstable only infrequently. Thus, differing degrees of instability do exist. Clearly, this complex of problems needs further clarification.

The ratios for *Yg* were larger than the remainder. In the case of 6x *(N. tabacum* × *tomentosiformis)* they were 4.7:1 and 4.4:1 for the two amphiploids synthesized from the races Cañamina and Tajma of *N. tomentosiformis*. These ratios were the largest ones listed in Table 1. Similarly, the ratio 14.8:1 obtained from a

N. tabacum × *otophora* amphiploid was the largest in Table 3. A comparable observation has also been made with respect to segregation for yellow green from 6x *(N. tabacum* × *sylvestris)* where a different subgenome was involved (GERSTEL and PHILLIPS 1958, and in prep.). The yellow green pentaploids in all these cases were throughout their lives conspicuously weaker than the normal sibs. In the original work on *Yg* segregation in *N. tabacum* NOLLA (1934) had obtained a highly significant deficiency of recessives in segregating populations. It is, thus, likely that the large segregation ratios in the pentaploid populations were due to a lessened viability of the yellow green segregants and not to a different behavior of the chromosomes carrying *Yg*.

SUMMARY

1. The original plan was to discover whether the chromosomes of *N. tomentosiformis* or those of *N. otophora* are more nearly homologous with one genome of *N. tabacum*.

2. The segregation ratios produced by the synthetic amphiploids *N. tabacum* × *tomentosiformis* and *N. tabacum* × *otophora* differed with 6x *(N. tabacum* × *tomentosiformis)* giving consistently the smaller ratios.

3. These results, taken at their face value, would favor *N. tomentosiformis* as the closer relation of tobacco, as far as chromosome homology is concerned, but judgment should be postponed because of the following complications:

4. Segregation ratios for 6x *(N. tabacum* × *tomentosiformis)* were always smaller than the 5:1 ratio expected for amphiploids synthesized from two very close relatives; in general the ratios were even smaller than the 3.7:1 ratio expected with chromatid segregation.

5. Segregation ratios for 6x *(N. tabacum* × *otophora)* varied over a wide range.

6. Only future work will indicate whether this variability is due to differences in the extent of differential affinity between chromosomes or of genetic instability.

7. Examples of somatic instability were described in the derivatives from 6x *(N. tabacum* × *otophora)*.

8. The yellow green locus gave larger segregation ratios than any other. Reduced viability of yellow green seedlings was the probable cause.

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LITERATURE CITED

AR-RUSHDI, A. H., 1956 Inheritance in *Nicotiana tabacum*. XXVI. Sterility genes from Tomentosae species. J. Genet. **54**: 9–22.

CLAUSEN, R. E., and D. R. CAMERON, 1944 Inheritance in *Nicotiana tabacum*. XVIII. Monosomic analysis. Genetics **29**: 447–477.

1950 Inheritance in *Nicotiana tabacum*. XXIII. Duplicate factors for chlorophyll production. Genetics **35**: 4–10.

GERSTEL, D. U., and L. L. PHILLIPS, 1958 Segregation of synthetic amphiploids in Gossypium and Nicotiana. Cold Spring Harbor Symposia Quant. Biol. **23**: 225–237.

GOODSPEED, T. H., 1954 *The Genus Nicotiana.* Chronica Botanica. Waltham, Mass.

GOODSPEED, T. H., and R. E. CLAUSEN, 1928 Interspecific hybridization in Nicotiana. VIII. The *sylvestris-tomentosa-tabacum* hybrid and its bearing on the origin of tobacco. Univ. Calif. Publ. Botany **11**: 127–140.

GREENLEAF, W. H., 1942 Genic sterility in *tabacum*-like amphidiploids of Nicotiana. J. Genet. **43**: 69–96.

LITTLE, T. M., 1945 Gene segregation in autotetraploids. Botan. Rev. **11**: 60–85.

MICHIE, D., 1953 Affinity: a new genetic phenomenon in the house mouse. Nature **171**: 26–27.

MOAV, R., and D. R. CAMERON, 1960 Genetic instability in Nicotiana hybrids. I. The expression of instability in *N. tabacum* × *N. plumbaginifolia.* Am. J. Botany **47**: 87–93.

NOLLA, J. A. B., 1934 Inheritance in Nicotiana. I. A study of the glaucous and yellow characters in *N. tabacum.* J. Agr. Univ. Puerto Rico **18**: 443–462.

SKALINSKA, M., 1935 Cytogenetic investigations of an allotetraploid Aquilegia. Bull. Int. Acad. Polon. Sci.; Classe Sci. Math. et Nat.; Sér. B. **1**: 33–63.

SMITH, H. H., and S. A. SAND, 1957 Genetic studies on somatic instability in cultures derived from hybrids between *Nicotiana langsdorffii* and *N. sanderae.* Genetics **42**: 560–582.

STINES, B. J., 1959 Diploidization in *Nicotiana tabacum.* A study of the yellow burley character. Master's thesis. North Carolina State College Library. Raleigh.

WALLACE, M. E., 1958 Experimental evidence for a new genetic phenomenon. Phil. Trans. Roy. Soc. London Ser. B. **241**: 211–254.

Literature Received

British Medical Bulletin. Blood Groups. Published by the British Council, London. Volume 15, No. 2, May, 1959. $3.25 per copy.

British Medical Bulletin. The Thyroid Gland. Published by the British Council, London. Volume 16, No. 2, May, 1960. $3.25 per copy.

Caldecott, Richard S., and Leon A. Snyder. A Symposium on Radioisotopes in the Biosphere. Center for Continuation Study, University of Minnesota, Minneapolis 14, Minnesota. 1960. 597 pp. $8.00 postpaid.

Cerebral Palsy Bulletin Supplement. A Proposed Standard of Nomenclature of Human Mitotic Chromosomes. National Spastics Society, 28 Fitzroy Square, London, England. Vol. 2, No. 3, 1960. 9 pp. $1.00 per copy.

Le Roy, Henri Louis. Statistische Methoden der Populations-genetik. Birkhauser Verlag Basel. Stuttgart. 1960. 397 pp. $6.50.

Poehlman, John Milton. Breeding Field Crops. Henry Holt and Co., New York. 1959. 427 pp. $8.50.

Research Highlights In Aging. U. S. Dept. of Health, Education, and Welfare. Superintendent of Documents, U. S. Government Printing Office, Washington 25, D. C. Public Health Service Publication No. 779. 1959. 52 pp. $.25 per copy.

Sheppard, P. M. Natural Selection and Heredity. Hutchinson and Co., London. 1958. 212 pp. 10s 6d net.

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GENETICS

A PERIODICAL RECORD OF INVESTIGATIONS
BEARING ON HEREDITY AND VARIATION

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The Second International Conference of Human Genetics will be held in Rome, Italy, on September 7–12, 1961. Information and registration forms may be obtained from the Secretariat of the Second International Conference of Human Genetics, Istituto "G. Mendel", 5 Piazza Galeno, Rome. The American Society of Human Genetics has been granted funds by the National Science Foundation and by the U. S. National Institutes of Health for the support of travel of a limited number of the American delegation to the Conference. The Secretary of the American Society of Human Genetics will mail application forms to members of the Society after December 1, 1960. Nonmembers may obtain forms from Professor C. P. Oliver, Department of Zoology, University of Texas, Austin. The amounts granted will depend on the number of applications, but probably will not exceed the amount required for air tourist round trip fare to Rome. It was decided by the Society that maximum travel grants would be awarded to persons invited by the Secretariat of the Conference to participate in Symposia and to applicants whose abstracts are approved by the Society Committee on Program Arrangements. Applicants, other than participants in Symposia, who plan to present scheduled scientific papers at the conference are to submit a carbon copy of their abstract to Dr. F. C. Fraser, Department of Genetics, McGill University, Montreal, Canada. The abstracts must arrive on or before April 1, 1961. Persons who will not present papers at the Conference may apply for partial travel assistance; the procedure is explained in the application form.

Molecular Basis of Neoplasia

"The Molecular Basis of Neoplasia" is the theme of the 1961 symposium on fundamental cancer research, sponsored by The University of Texas M.D. Anderson Hospital and Tumor Institute. Sessions for the 15th annual symposium were announced by Dr. Saul Kit, chairman of the meeting, for February 23, 24 and 25, 1961.

Sessions of the meeting will be devoted to nucleic acids, nucleic acids and proteins, mutation and protein structure, ribosomes and protein synthesis, controlling mechanisms and enzyme synthesis, and biochemical alterations induced by viral nucleic acids.

The symposium, which annually evokes international interest, will consist of 34 presentations by scientists from the United Kingdom, Austria, Israel and the United States.

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Programs and information on the meeting may be obtained by writing the Publications Department, The University of Texas M.D. Anderson Hospital and Tumor Institute, Texas Medical Center, Houston 25, Texas.

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